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CONTENTS

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EDITORIAL

The increasing demand for laboratory methods in the control and investigation of disease in man has produced a rapidly growing number of laboratories and specialists who are daily undertaking wider and more elaborate procedures in all four branches of pathology. Their work brings them into a special relationship with clinicians and patients, and it has become more and more difficult to extract from the wide range of journals covering these subjects the advances, experimental or established, which can be applied to the bedside.

The policy of the Association of Clinical Pathology and of the Editorial Board of the new Journal is, therefore, to provide under one cover a regular publication of such original articles, review articles, technical methods, and abstracts which will be of value to the practising clinical pathologist whose daily work must be orientated towards the elucidation of disease in man.

It is in no way intended that the Journal shall compete with the established publications, but rather that it shall augment them and in this way build a bridge between the experimental pathologist and the clinical worker.

A single number was published in July, 1945, by the Association of Clinical Pathologists, and so that these papers shall not be lost the contents are appended below

The Examination of Wounds for Clostridia, by Nancy J. Hayward.
Heterologous Agglutinins in Diagnostic Sera, by R. W. Fairbrother.
Liver Function Tests, by N. F. Maclagan.
The Examination of Bronchial Biopsy Tissues, by S. Roodhouse Gloyne.
Interpretation of the Endometrial Biopsy, by Oliver C. Lloyd.

THE LABORATORY DIAGNOSIS OF VIRUS INFECTIONS OF MAN: A REVIEW

BY

S. P. BEDSON

London Hospital and Medical College

The last fifteen years have seen a considerable advance in our knowledge of viruses. Admittedly it is still impossible to say whether or not viruses are living micro-organisms: there is even some doubt as to whether all those agents which we group together as viruses are of the same nature. But, interesting though these particular problems are, they have no direct bearing on the practical question of the diagnosis and control of virus disease, since for these purposes viruses can be regarded as micro-organisms. And the inability to answer these questions unequivocally does not mean that in other respects our knowledge of viruses has not made great strides. Many of the larger viruses have been stained and seen with the ordinary microscope, and the greater power of microscopic resolution obtainable by the use of ultra-violet light and a quartz optical system comprising a dark-ground condenser —an advantage which we owe largely to the work of J. E. Barnard in this country—has made possible the photography of these large viruses in an unstained and unaltered state, and has also brought some of the smaller viruses within the range of visibility. Furthermore, the introduction of the electron microscope, with its still greater powers of resolution, enables us to see even the smallest virus.

We are no longer confined to the use of animals for the cultivation of viruses. The adaptation of the technique of tissue culture to this purpose, and the use of the developing hen's or duck's egg—which we owe to the ingenuity of the American pathologist Goodpasture—have provided us with simple means for growing many of the animal viruses in the laboratory. And when we turn to the question of those serological reactions, the precipitin, agglutination, and complement fixation tests which have been of such invaluable service in the study of bacteria and bacterial disease, we find that, contrary to what was at one time thought, these methods are applicable to virus work. It is the purpose of this article

to consider some of this recent work and its application to the diagnosis of diseases of man of virus aetiology.

Microscopy

Demonstration of viruses by means of the microscope.—Sixty-one years ago Buist announced that he had succeeded in demonstrating particles with the appearance of minute cocci in smears made from vaccinial lesions (see Gordon, 1937); he concluded that these were the virus. As a recent American writer has remarked: "Buist was a man far ahead of his time; his contemporaries were not merely sceptical, they were not even interested." In 1904 Borrel published a paper on the virus of fowlpox, in which he showed that, by the use of Loeffler's flagella stain, minute coccal bodies could be demonstrated in smears of virulent material, and he considered these to be the virus. Two years later Paschen of Hamburg, using a very similar technique to Borrel, rediscovered the virus of vaccinia. Although this work did not pass unnoticed it did not receive general acceptance, in fact most bacteriologists adopted a sceptical attitude which it took nearly twenty years to dispel. The reason for this scepticism was due largely to the fact that the staining methods then employed, although producing admirable preparations in the hands of the expert, gave rise to artefacts bearing a remarkably close resemblance to stained virus particles, and the tendency to dismiss all these appearances as artefacts was all too readily adopted.

Since then, improved methods of staining and the devising of technical procedures for the separation of virus from the non-specific material in the crude suspensions of virulent material have made it possible to show that the particles one can stain and see in smear preparations are, in fact, virus. In this work, fractional centrifugation has played an important part. Saline suspensions of virulent

material are first centrifuged at a relatively low speed to throw down gross particles of tissue, and the resulting supernatant is then spun at a speed sufficient to deposit the virus. After removing the supernatant fluid, the deposit is resuspended in saline and centrifugation, first at a low speed and then at a high speed, is repeated. A deposit is finally obtained which consists very largely of particles resembling in size and staining properties those seen in the smears made from the original virulent material and thought to be virus, and the demonstration that the virulence of the original suspension has moved with the particles and that they react specifically with a known antiserum completes the proof that virus and particles are one and the same. There is, therefore, no doubt that the larger viruses can be seen with the microscope. They look like minute micro-organisms, and this conception of their nature finds support in the dark-ground photomicrographs that Barnard has produced of unfixed and unstained viruses.

Turning now to the practical application of this work to the diagnosis of virus disease, it is obvious that direct microscopy can be of use only in the case of the large viruses. Even here the limitations of the method are greater than in bacterial infections. The largest viruses are so much smaller than bacteria that they can be detected only if they are present in considerable numbers, and the occasions when their identity can be assumed because they have been seen in smear preparation are very few. However, the method is not without value and it finds its use particularly in the diagnosis of infections due to that group of large viruses which are distinguished from the others by staining by Castaneda's or Macchiavello's methods. For that matter, this group possesses other characteristics that mark it off from the rest: the viruses are larger, and when they multiply they present a regular sequence of morphological change, recalling those changes in size and shape which a bacterium often undergoes when multiplying. This group contains amongst others the agents of trachoma, inclusion conjunctivitis or inclusion blenorrhoea, lymphogranuloma venereum, and psittacosis. The use of microscopy in confirming the diagnosis of trachoma, particularly in its early stages, is well known. Halberstaedter and von Prowazek (1907) were first to demonstrate the characteristic cytoplasmic inclusions in the conjunctival epithelium in this condition, and the more recent work of Thygeson and Proctor (1935) has shown that the particles which occur both free and inside the cells and which are readily stained by Castaneda or Giemsa are, in fact, the virus.

Inclusion conjunctivitis.—Infection with the closely related virus of inclusion conjunctivitis is of greater interest to us, since it is of commoner occurrence than trachoma in this country. This virus is morphologically indistinguishable from that of trachoma; the exact relationship of the two, though obviously close, is yet to be determined. Like the trachoma virus, it is a parasite of man and is found producing infection of the conjunctiva and the mucous membrane of the cervix and the male urethra. Cells infected with this virus present inclusions which in situation, structure, and staining properties bear an extremely close resemblance to those of trachoma. They consist of masses of virus particles held together by inclusion material which is basophilic and scanty in amount. These inclusions differ, therefore, from what one might call the "typical" virus inclusion, in which the inclusion material is acidophil and present in such amount as completely to mask the virus. and it is this scantiness of inclusion material in the inclusions produced by the group of Castaneda-positive viruses which has made it so much easier to study and recognize their true structure. Like the other members of this group, the virus of inclusion conjunctivitis shows the sequence of developmental forms to which reference has already been made. The large forms, which are almost the size of staphylococci, have been called by Lindner (1910) "initial bodies," since in his opinion, they represent the initial stage in the development of the virus, a view which is fully supported by recent work. As the virus multiplies— and all appearances suggest that it does so by simple division—smaller and smaller forms are produced until, when the colony is fully grown and the cell is ready to break down and discharge its virus content, all the virus is in the form of elementary bodies.

Inclusion conjunctivitis occurs either as a neonatal infection, when it is acquired in precisely the same way as gonococcal ophthalmia, or in older children or adults, when it is often called swimming-bath conjunctivitis. Both forms are associated with inflammation of the conjunctiva and some discharge; scattered follicles may be present. Diagnosis is made by the microscopy of conjunctival scrapings suitably stained. and some idea of the incidence of the neonatal form can be gathered from a recent paper by Sorsby and others (1945), who found twenty-seven cases in 2% of all forms of conjunctivitis in infants.

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Cervicitis and urethritis.—Infection of the mucosa of the female genital tract is usually silent, but it can be revealed by the examination of stained smears of cervical scrapings. In the male, infection of the

urethra takes a subacute form which may become chronic and persist for months; this infection of the genital tract is transmitted by sexual intercourse and, although it undoubtedly occurs in this country, it appears to be uncommon.

Psittacosis.—The virus of psittacosis is another that can be recognized microscopically; and, although it is not suggested that direct microscopy is of value as a diagnostic procedure in human infections with this virus, it is undoubtedly of use in the case of infection of birds. It is often possible to find the virus in smears made from the spleen, which is commonly enlarged, or with material from the air sacs, where infection often resides, and so to make an early diagnosis.

Lymphogranuloma venereum.—Lymphogranuloma venereum virus, another member of the Castaneda-positive group, can also be detected in suitably stained smears of the pus from the inguinal bubocs which occur in the male and when the primary lesion is penile, or when it is situated in the anterior part of the genital tract of the female. Though possibly worth making, this investigation has not the same value in the diagnosis of lymphogranuloma venereum as isolation of the virus, the Frei test, or the complement fixation reaction.

Smallpox.—As a further example of the diagnostic value of the microscopical demonstration of virus, mention might be made of its use in smallpox. This virus is of a size readily visible under the microscope, and a number of staining methods are available for its demonstration, some of which, such as the alkaline methyl-violet method of Gutstein (1937), are quite simple. The virus is present in quantity in the skin lesions and, as van Rooyen and Illingworth (1944) have shown, microscopic examination of suitably stained smears made from scrapings obtained from the base of papules or vesicles can be of considerable diagnostic value. In the cutaneous lesions due to herpes febrilis, zoster, or varicella, the virus particles, though present and staining by the methods applicable to variola, are neither so numerous nor so large as variola virus. Van Rooyen and Illingworth used Paschen's staining method. Commenting on this diagnostic procedure, Downie (1946) states a preference for Gutstein's stain and points out that smears made from pustular lesions or crusts are unreliable, that a negative result does not exclude smallpox, and that the test would not differentiate between the lesions of smallpox and vaccinia.

Demonstration of specific histological changes.—Quite apart from the possibility of demonstrating the virus itself, there are, in many virus diseases, demonstrable histological changes in the infected cells which are sufficiently distinctive to be of diagnostic value. These are the inclusion bodies found in the cytoplasm or the nucleus of the affected cells, bodies of varying size, usually homogeneous in appearance and acidophil in staining reaction, which may come to occupy the major part of the nucleus or a considerable part of the cell's cytoplasm. Doubt still exists as to the exact nature of the nuclear inclusions, but it has been shown that many cytoplasmic inclusions are virus colonies, masses of virus embedded in a matrix of homogeneous material. It is this latter material which gives to many of these inclusions their appearance of homogeneity and affinity for acid dyes. In the case of the cytoplasmic inclusions produced by the Castaneda-positive viruses, inclusion material is in insufficient quantity to mask the virus particles, and these can usually be readily seen either in Castaneda- or Giemsa-stained preparations. Even where the inclusions appear homogeneous when fixed and stained, it is still possible, as Barnard has shown in the case of ectromelia, to demonstrate the true nature of the inclusion by using dark-ground illumination. The difference in refractility of virus and inclusion material shows up the former as bright dots.

At one time it was thought that only infection with a virus could produce inclusion bodies, but it is now known that very similar appearances can be produced experimentally by other means. This applies particularly to nuclear inclusions. None the less, the finding of inclusion bodies can be taken as presumptive evidence of a virus infection, and in some instances their demonstration has diagnostic value. The finding of Negri bodies, the acidophil cytoplasmic inclusions produced by rabies virus in nerve cells, confirms the diagnosis of suspected rabies in the dog. And mention has already been made of the demonstration of the Halberstaedter-Prowazek bodies in trachoma and the similar inclusions in inclusion conjunctivitis, cervicitis, and urethritis as diagnostic procedures. Paul's test for smallpox, which depended on the production of lesions in the rabbit's cornea characterized by cytoplasmic inclusions, has been largely superseded by the demonstration of the infective agent in the skin lesions either by direct microscopy or by the complement fixation test.

Isolation of the Virus

If the application of microscopy to the diagnosis of virus infections has been dealt with at some length this has been done deliberately in order to

emphasize that, contrary to common belief, many viruses are sufficiently large to be seen and recognized by means of the microscope. The limitations of this procedure have not, however, been lost to sight, and, in virus infections as in any other infective process, the aim of the laboratory should be the isolation and identification of the infective agent. Unfortunately this is less easy to achieve than in bacterial disease. Not only is the isolation and identification of a virus a lengthy and often difficult procedure, involving the infection of animals or embryonated eggs, the study of the histological lesions produced by this infection, and the application of serological tests usually in the form of the neutralization reaction, but there are quite a number of viruses infecting man for which a suitable experimental animal has not been found. The viruses of zoster, varicella, and infective hepatitis exemplify this: to them, man alone would seem to be susceptible. Or it may be that the only susceptible species of animal is so expensive as to preclude its routine use; it is this factor, probably more than any other, which has delayed our understanding of poliomyelitis and its epidemiology. Even where a virus can be isolated by the use of the incubated egg or an inexpensive animal, the time taken to establish and identify it is too long to make the procedure of immediate diagnostic importance. That does not mean that every attempt should not be made to isolate the agent of a suspected virus infection, because this should, of course, always be done where the diagnosis is in doubt. The following examples illustrate some of the commoner uses of this form of investigation.

Egg inoculation.—It is at times important to know whether an outbreak of clinical influenza is due to one of the known influenza viruses and, if so, which one. This might be necessary, for instance, at the commencement of an epidemic when prophylactic immunization is contemplated; and, though at one time the ferret would have been chosen as the most suitable animal for the purpose, probably the incubated hen's egg would now be the choice. Hirst (1942 and 1945) has found the injection of naso-pharyngeal washings into the amniotic sac of thirteen-day eggs slightly superior to ferret inoculation for the isolation of influenza A virus, an observation confirmed by Burnet and others (1942). And Beveridge and others (1944) employed the same method successfully for the isolation of influenza B virus. The combination of penicillin and sulphadiazine with the crude washings does away with the necessity for filtration. The embryonic membranes of the developing hen's or duck's egg—the amniotic,

allantoic, and yolk sacs and the chorio-allantois—provide admirable means for the cultivation of a number of viruses to which man is susceptible; in addition to influenza A and B, the viruses of herpes simplex, psittacosis, lymphogranuloma venereum, mumps, measles, lymphocytic meningitis, and smallpox can all be grown on one or other of these membranes. Incidentally, North and others (1944) utilized inoculation of the chorio-allantois with suspensions of skin crusts in the diagnosis of smallpox, a procedure the value of which has been fully confirmed by Downie (1946) and Downie and Dumbell (1947).

Value of animal inoculation.—Invaluable though the egg technique has proved in virus work, it has by no means superseded the use of experimental animals, for there are occasions when only the latter are suitable to the work in hand. This is true, for instance, of poliomyelitis, where inoculation of the monkey is our only means of demonstrating the presence of the virus. Even in those cases where the virus sought will infect both eggs and animals it is advantageous to use both; it increases the chance of success and it may well be that the disease produced in the animal is more distinctive than the changes resulting from infection of the egg. The latter may, in fact, be so inconspicuous as to make their detection difficult and uncertain, as in infection of the chorio-allantois with the virus of lymphocytic chorio-meningitis. Or, as recent work on rinderpest has shown (Shope and others, 1946), a virus may multiply within the developing egg without producing any detectable naked-eye or histological changes or even death of the embryo.

Meningitis.—So where both egg and animal are available for our purpose we use both. This is true, for instance, in the investigation of acute aseptic meningitis, a syndrome due to a number of viruses, some of which have been identified. Of these, the virus of lymphocytic choriomeningitis of Armstrong and Lillie is responsible for about one-third of cases. At any rate, that would appear to be so in North America, though one has the impression that it is a less frequent cause of benign meningitis in this country. Other viruses which have been found producing this form of meningitis are the pseudo lymphocytic choriomeningitis virus of MacCallum, Findlay, and Scott (1939) and the virus of lymphogranuloma venereum. Infection with the last-named virus may rarely present as a meningitis (Sabin and Aring, 1942), and the virus of MacCallum and Findlay has been isolated on only *two* occasions; more than half the cases of this condition are due to a virus or viruses yet to be identified. The three

known causal agents are to be found in the cerebro-spinal fluid, and all these are pathogenic for the mouse and guinea-pig. Wherever the diagnosis of acute aseptic meningitis is in question, cerebro-spinal fluid collected as early in the disease as possible should be injected intracerebrally in mice and subcutaneously in the thigh of guinea-pigs. Should any of the three viruses be present, encephalitis may develop in one or more of the mice, and the virus so isolated can be established and studied. The virus of Armstrong and Lillie (1934) will often infect the guinea-pig, producing a generalized infection with pyrexia, wasting, and salivation, the animal dying usually in from nine to sixteen days. Should the case of meningitis prove to be tuberculous, and in the early stages of this condition the differential diagnosis from benign lymphocytic meningitis of virus origin may well arise, the mice will remain well whereas the guinea-pigs will develop tuberculosis.

Lymphogranuloma venereum.—In addition to producing an encephalitis when inoculated intracerebrally in mice, lymphogranuloma virus also produces a local lesion when injected sub-cutaneously in the guinea-pig's thigh, and an enlargement of the inguinal lymph nodes occurs; the lesions can be examined histologically; and, by further passage in animals and eggs, the virus can be established and identified. The use of monkeys in such an investigation has not been mentioned because they are expensive and not essential, though, if available, one or possibly two might be inoculated intracerebrally. And it has been assumed that eggs, if available, will be used in parallel with the animals.

Psittacosis group.—Another example of the use of animal inoculation, in addition to eggs, in the diagnosis of virus infection is provided by diseases caused by viruses of the psittacosis group. Human infection with psittacosis virus is often associated with some degree of pneumonia, and either sputum or lung puncture material can be used for animal inoculation, though in psittacosis pneumonia sputum is often scanty. The mouse is the animal usually employed; and, if sputum is the material chosen, the presence of bacteria, particularly the pneumococcus, may introduce a complication. Though the bacteria can be got rid of by filtration, this also reduces the amount of virus, and a better procedure is to protect the mouse against the bacteria by means of sulphonamides, to which psittacosis virus is insensitive, or relatively so. This procedure has the additional advantage, if the mice have been inoculated by the nasal route, of preventing a lighting up of latent infection of the mice with the virus of Nigg (Nigg and Eaton, 1944), present in many mouse stocks. This virus belongs to the Castaneda-positive group and is fortunately susceptible to sulphonamides. And, in connexion with the use of mice for the isolation of psittacosis virus, it is important that both the intranasal and intraperitoneal routes of inoculation should be employed, because certain strains of psittacosis virus produce only an inapparent infection when introduced into the peritoneal cavity. This is true of pigeon strains, and here it might be pointed out that the host range of this virus is much more extensive than was at one time thought. In addition to birds of the parrot tribe, certain species of finch, fulmar petrels, pigeons, the domestic fowl, and ducks have been found to suffer from natural infection with psittacosis virus and to be responsible for human infections.

Serological Investigation

It is still far from being widely appreciated that those serological tests which are in daily use in the identification of bacteria and the diagnosis of bacterial disease are equally applicable to viruses. Virus workers themselves have been to some extent responsible for the tardy recognition of this fact. There is no need here to consider the reasons for this; it is sufficient to note that it is now well established that the precipitin, agglutination, and complement fixation tests are all available for the investigation of viruses and the diseases they cause. And, because it is more difficult to obtain a suspension rich in virus than is the case with a bacterium, the complement fixation test is the serological reaction which has been found most suitable for virus work, since, as Merril has shown (1936), this test has a lower antigen threshold than the precipitin or agglutination reactions. A serological test could be used in the diagnosis of a virus infection either for the purpose of demonstrating the presence of virus in the infective material or for the detection of antibodies formed in response to the infection.

Demonstrating virus in infective material.—As an example of the former we have the use of the complement fixation test in the diagnosis of smallpox. Craigie and Wishart (1936) have shown that this is a reliable test for the presence of variola antigen in the vesicle fluid or in an extract made from the crusts of older skin lesions; the serum employed is one made against vaccinia virus in the rabbit. Tulloch (cited by Downie, 1946) has had wide experience of this test in the outbreaks of smallpox which occurred in Scotland in the war

of 1939–45; he considers the test to have a high diagnostic value. This opinion is shared by Downie (1946), who found that vesicle fluid or crusts from twenty-two cases suspected of being smallpox on clinical or epidemiological grounds gave a positive result; his material came from twelve different outbreaks. Similar material from other conditions, such as varicella, zoster, or septic skin rashes, gave a negative reaction. He concludes that this is the most valuable single laboratory test available for the diagnosis of smallpox, an opinion which seems fully justified by the evidence.

This is one of the few examples, however, of this particular use of serological reactions in the diagnosis of human virus infections. It has, at times, been applied, in conjunction with animal inoculation, for the differentiation of zoster from herpes simplex, using a known positive convalescent zoster serum and the fluid from the vesicular eruption (Bedson, unpublished observations).

Detection of antibodies.—But, generally speaking, the concentration of antigen in infective material from virus infections is too low even for its detection by the complement fixation reaction, and it is for this reason that the indirect use of such a test (that is, for the demonstration of specific antibody in the patient's serum) is of so much wider application as a diagnostic procedure. It would be impossible here to give a comprehensive survey of this use of the complement fixation test; a few illustrative samples will have to suffice.

Lymphocytic choriomeningitis.—In the course of infection with the virus of lymphocytic choriomeningitis, complement fixing antibodies are developed (Smadel and others, 1939) for the soluble antigen of this virus. Although these antibodies have probably nothing to do with immunity to this virus, they are specific and their presence diagnostic (Smadel and others, 1939; Lépine and Sautter, 1938). The soluble antigen is present in considerable amount in the tissues of the experimentally infected animals, and a suspension of the spleens of guinea-pigs succumbing to lymphocytic choriomeningitis provides a satisfactory antigen which remains stable when stored in the refrigerator for months or even a year or more. It is important to note that a freshly made spleen suspension may give false reactions, it should be allowed to age for two to three weeks before use. Although the American and French work already referred to leaves no doubt as to the specificity and diagnostic value of this test, limited experience of its use in this country has been disappointing (Bedson, unpublished findings). Dur-ing the war a number of sera from cases of benign aseptic meningitis have been examined · without obtaining a single positive result, and, although in some of these cases this was probably due to their not being caused by the virus of Lillie and Armstrong, there were two from which this virus was isolated by mouse inoculation.

Influenza.—The complement fixation test can also be applied to the diagnosis of influenza (Smith, 1936; Hoyle and Fairbrother, 1937; Fairbrother and Hoyle, 1937; Francis and Magill, 1937; Tulloch, 1939), but since antibodies to influenza virus are to be found in detectable though low titre in the blood of most adults and older children, diagnosis depends on demonstrating a significant increase in these antibodies, and that makes the findings mainly of retrospective interest. This being so the neutralization test, which takes so much longer to do, might equally well be employed, and in the past it is the neutralization test which most workers have used. It may be, of course, that the Hirst phenomenon of agglutination of the red cells of the domestic fowl by influenza virus (1941), which is specifically inhibited by influenzal antibody and can be used as a method of antibody titration, will eventually largely replace both complement fixation and neutralization tests.

Virus agglutination of red cells.—The subject of virus agglutination of red cells, which, since Hirst's original work, has been the object of much study, principally by Burnet and his colleagues in Australia, leads to mention of a recent observation from Burnet's laboratory. This is not the place for any detailed consideration of all the interesting work done in this field; suffice it to say that the phenomenon of red-cell agglutination is not confined to the influenza virus and chick cells; other viruses will do this, and the red corpuscles of certain species of mammal, including man, are agglutinated as well as chicken red cells. The absorbed virus responsible for the agglutination is liberated after a few hours; this fact, incidentally, is utilized in the concentration of the influenza viruses in the preparation of influenza vaccine.

The recent observation of Burnet and Anderson (1946) to which it is desired to draw attention is that human red cells which have been agglutinated by the virus of Newcastle disease of fowls and from which the virus has been eluted have become susceptible to agglutination by the majority of sera from recent cases of glandular fever. This work is still in its initial stages. The evidence produced by Burnet and his colleague suggests that the change in

the cells is due to the absorption of antigenic material, other than virus, produced by Newcastle disease virus when growing in chick embryo cells. Burnet (1946) has also shown that human red cells treated with egg-grown mumps virus provide a sensitive test for mumps antibody in human sera. Although much remains to be done, this work opens up such exceedingly interesting possibilities that it seemed well worth mentioning.

Psittacosis-lymphogranuloma venereum group.—It is perhaps in the diagnosis of infections with the viruses of the psittacosis-lymphogranuloma venereum group that the complement fixation test has been most widely applied. This test has been used in the diagnosis of psittacosis in man (Bedson, 1935 and 1937) for eleven years or more, and the work of Meyer and his colleagues and others in America has confirmed its value. By means of this test support was given to the findings of Haagen and Mauer (1939) that cases of an atypical pneumonia occurring in the Faroe Islands were due to psittacosis or some very closely related virus, and it was used to show that a similar clinical condition in Iceland had a like aetiology (Bedson, 1940). Infection in these cases originated in fulmar petrels. Some involvement of the lung is of frequent occurrence in human infections with psittacosis virus, and it has long been recognized that the resulting pneumonia was unlike bacterial pneumonia in its clinical signs and histological picture. It was not, therefore, very surprising when Eaton and others (1941) showed that some cases of the condition known as primary atypical pneumonia, which in the recent war assumed almost epidemic proportions in this country and elsewhere, were due to psittacosis virus. This observation was based on isolation of the virus from sputum or lung material and the demonstration of a positive complement fixation test with the patients' sera. Subsequent work has shown that an appreciable proportion of cases presenting this syndrome, which Reimann (1946) quite rightly prefers to name "virus pneumonia," are due to viruses belonging to the psittacosis group. Smadel (1943), for instance, found that some 15 per cent of forty-five cases of so-called primary atypical pneumonia were probably due to a psittacosis virus, and, in a serological survey made of this condition in England during the war, nine cases out of 120 had a very high or rising titre of antibody for psittacosis virus (Bedson; unpublished findings).

The more extensive use of the psittacosis complement fixation test resulting from these investigations has shown that the mere presence of such an antibody in the serum of an individual does not necessarily mean active infection with a virus of this group. As in the use of any serological test for antibody as a diagnostic procedure, the demonstration of a rising titre of antibody is necessary to postulate active infection. Smadel (1943) rightly insists that only a fourfold rise in psittacosis antibody should be accepted as evidence of active infection, and with this the writer would fully concur. The recent work in America showing that the viruses belonging to the Castaneda-positive group are closely similar in antigenic structure has introduced a further complication. Rake and others (1941) found that sera from patients infected with the viruses of lymphogranuloma venereum and the psittacosis group all cross-reacted when tested by the complement fixation test with these viruses. They further showed that cases of atypical pneumonia due to a psittacosis virus might give a positive Frei test. And the demonstration by Rake and others a year later (1942) that the sera from cases of trachoma and inclusion conjunctivitis may fix complement with the antigens of lymphogranuloma virus suggests that these two viruses also share antigens with the psittacosis-lymphogranuloma group. There is further evidence obtained from cross-immunity experiments in animals confirming the close relationship between the viruses of the psittacosis group—parrot psittacosis, pigeon psittacosis or ornithosis, and the virus isolated by Eaton and others from atypical pneumonia—and the virus of lymphogranuloma venereum. How close this relationship will prove to be remains to be seen, but the immediate practical bearing of this work is that the serological tests which have been accepted in the past as specific for psittacosis and lymphogranuloma venereum can no longer be so regarded. And the Frei test, as one might expect, would appear to be no more specific than the serological tests. Though this does not mean that these tests are without value, it does emphasize the importance, which of course always existed, of invariably interpreting them in conjunction with the clinical findings.

General Remarks

This account of the part which the laboratory can play in the diagnosis of virus infections is far from complete. Only the more important examples of this have been mentioned, and they have been given shorn of much detail and supporting evidence. It is hoped, however, that this paper will have shown that the laboratory can be of help, not just occasionally by making some special investigation, but in an everyday routine way.

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MENINGOCOCCAL MENINGITIS WITH PARTICULAR REFERENCE TO EPIDEMIOLOGY AND PATHOGENESIS

BY

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In this country cerebrospinal meningitis (fever) is an endemic disease found mainly in children and adolescents; local outbreaks occur, but they are rare and usually involve few individuals. The disease has, however, a marked predilection for war-time conditions; relatively extensive outbreaks occurred during the 1914–18 war, and again to an even greater extent during the 1939–45 war. These outbreaks involved individuals of all ages, including Service personnel, but it is interesting to note that, even though there was a great increase in the total number of cases notified, these tended to occur singly and an epidemic, in the generally accepted sense, did not develop.

Although such general factors as the enhanced virulence of the meningococcus and the resistance of the individual are well recognized as playing an important role in the epidemiology of the disease, a precise explanation of the peculiar nature of the outbreaks is still lacking. A popular theory is that outbreaks of cerebrospinal meningitis result from a widespread meningococcal infection of the nasopharynx in the general population (Ministry of Health Memorandum, 1940). Many workers accept the hypothesis developed by Dopter (1921) that meningococcal infection occurs in three progressive stages: (1) catarrhal, (2) septicaemic, and (3) meningitic; in the majority of cases the infection is arrested at the catarrhal stage and the occurrence of meningitis is a rare phenomenon (Lundie and others, 1915; Herrick 1918; Murray, 1929; Brinton, 1941). The evidence offered to support this hypothesis is, however, inconclusive and unconvincing. Furthermore this theory does not provide a solution of many problems presented in the various outbreaks, and it seemed desirable that the question should be re-examined in the light of information gained during the 1940–42 outbreak.

During the past seven years an opportunity has been available of studying the disease under Service and civilian conditions during both so-called epidemic and interepidemic periods and it was possible to direct attention particularly to the epidemiology and pathogenesis of the disease. The results are discussed below; they do not support the above hypothesis and an alternative is offered.

Technique

Cerebrospinal fluid.—Samples of cerebrospinal fluid were centrifuged as soon after collection as possible for 5 or 10 minutes; the supernatant fluid was decanted and the deposit seeded heavily on to Loeffler serum slopes and blood-agar plates, films were then made and the remainder of the deposit added to 10 ml. of glucose-broth, usually containing a small amount of para-aminobenzoic acid. Incubation was carried out at 37° C.; the blood-agar plates were usually placed in an atmosphere containing approximately 10 per cent carbon dioxide.

Nasopharyngeal swabs.—Material was collected from the nasopharynx by means of the West swab, which was pressed and then stroked firmly against the mucous membrane. Cultures were prepared on blood-agar plates; after overnight incubation under ordinary atmospheric conditions, suspicious colonies were subcultured on to Loeffler slopes for further investigation; the plates were invariably incubated a further twenty-four hours and re-examined.

Blood cultures.—Ten ml. of blood were collected by venepuncture; 2 ml. were added to a small amount of trypsin broth before mixing with 10 ml. agar, 2 ml. to Robertson's meat medium, and the remainder was placed in 25 or 50 ml. of glucose broth. Incubation at 37° C. was continued for seven to fourteen days before a negative report was given, subcultures on Loeffler serum slopes being made after forty-eight hours and later as indicated.

The identification of the meningococcus was carried out by fermentation tests with sucrose, maltose, and

glucose in Hiss's serum water and finally by agglutination tests using Standard Group sera. The suspensions for the agglutination tests were made by emulsifying the growth from a Loeffler slope in normal saline and adjusting to a light opacity; the tube method with incubation at 55° C. was used. Standard Group sera, from Army and M.R.C. sources, were used throughout.

Preliminary tests were frequently carried out by the slide agglutination technique; useful information was obtained when the strains readily agglutinated, but this method proved unsatisfactory with relatively inagglutinable organisms.

Scope of the Investigation

Material was collected from various sources during this investigation, particularly from cases, close contacts of cases, and members of the general community (Table I).

TABLE I

SOURCE OF MATERIAL

Type of case				Number
Meningitis ⟨service	..	..	..	105
civilian	..	..	..	47
Purulent conjunctivitis	..	..	..	2
Chronic septicaemia	..	..	..	3
? Carrier ⟨close contacts	..	..	..	144
general community	..	..	..	530

Cerebrospinal fluid only was examined from the civilian cases of meningitis, but the Service cases were investigated in greater detail. Cerebrospinal fluid was examined from all, while blood cultures and nasopharyngeal swabs were collected from a limited number. The search for carriers was carried out by the collection of nasopharyngeal swabs. Close contacts (Service personnel) were considered to be individuals occupying beds adjoining the case or all occupants of small rooms or tents in which the case was sleeping (War Office Memorandum, 1940). While such a definition did not embrace all close contacts, it did ensure that all members of this group had been exposed to the nasopharyngeal discharge of the patient a short time before the collection of the swab.

Clinical Course and Therapy

The various clinical manifestations of meningococcal infections have been described in detail by Rolleston (1919), Priest (1941), and Brinton (1941). The cases seen during this investigation followed the general pattern. The cases of meningitis during the 1940–42 outbreak were mainly of a severe type, the initial symptoms being headache, neck rigidity, vomiting, and malaise. Some examples of the fulminating meningeal type were seen; these within a few hours of the onset of the disease exhibited mental confusion and delirium. During the later stages of the outbreak the mild abortive form of the disease was occasionally encountered. No examples of adrenal involvement (Waterhouse-Friderichsen syndrome) or of encephalitis (Banks and McCartney, 1942) were seen.

Intensive chemotherapy with a sulphonamide, usually sulphapyridine, was begun immediately the diagnosis was established on the lines recommended by Banks (1939). Serum was not used. The usual practice was to examine the cerebrospinal fluid before chemotherapy was instituted; the fluid was invariably turbid and meningococci generally demonstrable.

In the fulminating types of disease the soluble form of the sulphonamide was administered by the intravenous route.

The results were excellent; in the group of 105 Service cases there was only one death. This is very much lower than the general mortality rate for the disease, but it must be appreciated that the cases were adults of military age and that therapy was usually instituted early in the disease; it is well known that the mortality rate is highest at the extremes of life (Jubb, 1943; Beeson and Westerman, 1943).

Several cases of chronic meningococcal septicaemia were suspected on clinical grounds, but in only three instances was it possible to confirm the diagnosis by blood culture. When this was accomplished sulphonamide therapy was instituted with the anticipated dramatic effect.

Bacteriology

Meningococci isolated during this investigation generally behaved in the characteristic manner which was described in detail by Murray (1929). A few interesting features were, however, observed, and these merit special consideration.

The standard solid media used throughout the investigation were 6 per cent blood-agar plates and Loeffler serum slopes. Both media gave a satisfactory growth but it was observed that, under ordinary atmospheric conditions, strains isolated in pure culture directly from the cerebrospinal fluid grew readily on the Loeffler serum slopes in screw-cap bottles, while growth on blood-agar plates was not infrequently poor; incubation of the blood agar plate in 10 per cent carbon dioxide, however, gave a luxuriant growth. It therefore follows

that, if blood-agar is used for the isolation of the meningococcus from cerebrospinal fluid, incubation should be carried out in an atmosphere containing carbon dioxide.

Biochemical tests were limited to the fermentation of glucose, maltose, and sucrose in serum-water. The great majority of strains gave consistent results, glucose and maltose being fermented but not sucrose; irregularities were occasionally found.

The agglutination tests followed the generally accepted rule that strains isolated during an "epidemic" period agglutinate readily with standard sera, but during "non-epidemic" periods inagglutinable strains tend to appear. During the main 1940–42 outbreak, cerebro-spinal fluid strains agglutinated readily with the Standard Group sera; many gave clear-cut agglutination only with Group I serum, but some did not exhibit any marked antigenic specificity and agglutinated also to varying degrees with Group II serum. Many indications of the complex antigenic structure of the meningococcus were obtained during this investigation, but it was not possible to examine this problem. A simple classification by means of Standard Group sera only was attempted.

When the large outbreak had subsided and infrequent sporadic cases only were reported, a different serological picture was seen. Thus during the 1946–7 winter several severe cases, mainly in infants, were investigated; meningococci were isolated from the cerebrospinal fluid but these tended to agglutinate poorly, usually giving a slight reaction only with the Group II serum; one strain proved to be inagglutinable with the sera available (Table II).

Nasopharyngeal swabs gave similar results; strains isolated during the main outbreak were more easily grouped than those obtained during the non-epidemic period. Throughout the investigation Group II strains were predominant.

Epidemiology

The epidemiology of cerebrospinal meningitis presents several unusual features for which no adequate explanation has been offered. The marked predilection of the disease for war-time conditions has long been recognized, and therefore the sudden widespread outbreak of the disease at the beginning of 1940 was not unexpected. It is interesting to note that the incidence of the disease in this country during 1940 reached the unprecedented total of 12,771 cases; this figure is actually greater than the total figures for the 1914–18 period. There was not, however, an epidemic in the generally accepted sense of the term; cases were widely distributed, occurred sporadically, and involved individuals of all ages. Fortunately the sulphonamides proved excellent therapeutic agents and some of the serious problems of 1914–18, such as

the high mortality rate and long periods of hospitalization, did not arise.

During the 1914–18 outbreak special research teams carried out extensive investigations of the disease and the following important fundamental points were established: many normal individuals harbour the meningococcus in their nasopharynx; cases of cerebrospinal meningitis develop almost entirely from contact with a carrier (instances of infection from other cases of the disease occur but they are relatively rare); the disease is transmitted by droplet infection, and consequently overcrowding and poor ventilation play prominent roles in the spread of infection.

The precise significance of the carrier is uncertain. Glover (1920) claimed that a carrier rate of over 20 per cent in a community was not only an index of overcrowding but also a warning of an impending outbreak of meningitis, and he stressed the importance of the search for carriers as a prophylactic measure. Subsequent observations have, however, failed to confirm this rather extreme view. Dudley and Brennan (1934) in a naval garrison found a carrier rate of 13 per cent associated with 11 cases of meningitis, while at a later period the carrier rate was 55 per cent but no case of meningitis developed. Other workers have also found carrier rates over 20 per cent without any increased incidence of meningitis (Straker et al., 1939). It is, therefore, obvious that the development of meningitis in an individual is dependent on factors other than the presence of carriers in the community, and there is little doubt that these are closely concerned with (1) the virulence of the meningococcus, and (2) the resistance of the host.

Virulence of meningococcus.—The absence of suitable animals has prevented a detailed study of this problem and it has been necessary to depend mainly on indirect serological tests for information. During the 1914–18 outbreak four serological types were identified (Gordon and Murray, 1915); these types were not sharply demarcated and considerable antigenic overlapping occurred. In view of this other workers (Griffith, 1918; Scott, 1918) considered that the most practical serological classification was into two main groups—Group I embracing types I and III, and Group II comprising types II and IV. It was generally recognized that inagglutinable strains were particularly prominent in the nasopharynx during interepidemic periods.

The use of Group sera is now the accepted method of subdividing meningococci, but knowledge of the antigenic structure of the meningococcus is still incomplete and irregularities are not uncommon.

In the 1914–18 outbreak, the majority of cases were caused by types I and II, but since that time there has been a close relationship between Group I strains and major outbreaks. It is therefore not surprising to find that, while Group II strains have predominated in carriers, some 90 per cent of strains isolated in this country from cerebrospinal fluid during the 1940–42 outbreak belonged to Group I (Harries, 1942; Ministry of Health Report, 1946).

During this investigation there was striking serological difference in the strains isolated during the 1940–42 outbreak and those isolated from the infrequent cases seen during the 1946–7 winter (Table II).

TABLE II

SEROLOGY OF MENINGOCOCCI ISOLATED FROM CEREBROSPINAL FLUID

	1940–2	1946–7
Group I	86	1
Group II	3	9
Inagglutinable	1	1
Total	90	11

The close association of Group I strains with major outbreaks or "epidemics" suggests strongly that such strains are more virulent than those of Group II. It is, however, the general experience that there is little or no difference in the severity of the disease produced by these strains. It must be appreciated that normal cerebrospinal fluid is an excellent culture medium for most bacteria and, provided a sufficient number is introduced, multiplication will occur irrespective of the intrinsic pathogenicity of the organism.

An important difference in the two serological groups of meningococci is therefore the greater facility with which Group I strains are able to pass from the nasopharynx to the cerebrospinal fluid. The factors concerned with the virulence of the meningococcus are obscure, but they appear to be associated with the surface, probably the capsule, of the organism. The production of toxic substances by the meningococcus has been demonstrated by various workers (Branham, 1940); knowledge of these is, however, vague, and it has not been proved that they play any part in the passage of the organism to the subarachnoid space.

The low incidence of meningitis in spite of the wide distribution of the meningococcus indicates clearly that the passage from the nasopharynx to the meninges' is not readily accomplished. An important reason for this is the barrier to invasion presented by the nasopharynx; the responsible factors consequently merit close consideration.

Resistance of the host.—During the 1939–45 war conditions favoured the wide dissemination of the meningococcus, particularly in the Services, as overcrowding was common and ventilation of rooms often inadequate as a result of stringent black-out precautions. Although there was a greatly increased incidence of meningitis during the main outbreak of 1940–42, the majority of cases were single and widely distributed, having no direct contact with each other. Most individuals would, therefore, appear to possess a high degree of resistance against the meningococcus, and the factors concerned in this have been the subject of much speculation.

Many advocate the theory that infection with the meningococcus occurs in three progressive stages: (1) catarrhal, (2) septicaemic, and (3) meningitic; the common form of the infection is a nasopharyngitis, and only in relatively few cases does the organism penetrate the pharyngeal barrier to reach the blood stream, from which the meninges are attacked. The nature of this pharyngeal barrier has, however, never been satisfactorily explained.

The so-called catarrhal stage is obviously the one of greatest epidemiological' significance in view of the potential danger to the community. It is consequently important that the criteria on which this theory is postulated should be critically examined.

The main arguments put forward to support this theory are: (1) the isolation of the homologous meningococcus from both the nasopharynx and cerebrospinal fluid of early cases (Flack, 1917), (2) the history of premeningitic sore-throat or catarrh given by some cases, and (3) the widespread distribution of carriers.

These views have not passed unchallenged, and the following points have been raised in opposition: (1) the failure of many workers to isolate regularly the meningococcus from the nasopharynx of early cases of meningitis (Andrewes and others, 1916); it is also interesting to note in this connexion that Fildes and Baker (1918) reported the occurrence of meningitis in twenty-six naval ratings although their nasopharyngeal swabs had been negative at varying periods from two to seventy-five days before the onset of symptoms, and concluded that cases can seldom be carriers before the onset of the disease; (2) a history of sore-throat or catarrh is frequently unobtainable from cases or carriers (Foster and

Gaskell, 1916; Worster-Drought and Kennedy, 1919); (3) cerebrospinal meningitis occurs mainly during the early months of the year when upper respiratory infections are common, and their association with meningitis is likely to be coincidental (it has even been suggested that this non-specific catarrh might constitute a predisposing factor to the development of meningitis); (4) case-to-case infection is extremely rare; the source of infection is almost invariably an undetected carrier; (5) in the majority of instances the carrier state is temporary.

As a reply to these arguments protagonists of the catarrhal theory claim that the local meningococcal infection is generally mild and transient, giving rise to little or no personal discomfort, but clinical and bacteriological confirmation is seldom produced.

There is general agreement that in every case of meningitis the portal of entry of the meningococcus is the nasopharynx. There is, however, a lack of convincing evidence to indicate that the production of a nasopharyngitis, even in a mild form, is an essential stage in the development of meningitis. It must be pointed out that the examination of nasopharyngeal swabs from early cases is rarely carried out, and knowledge of this important aspect of the subject is consequently limited.

During this investigation the validity of the "catarrhal" theory was examined by the collection of swabs from the following groups of the community:

1. *Close contacts.*—These were soldiers sleeping in beds adjoining the case, or all occupants in cases arising in tents or billets. All had had ample opportunity of receiving the upper respiratory flora from the cases as overcrowding was often present and ventilation, particularly at night, usually poor; the swabs were collected within a short time, often a few hours, of the removal of the case.

2. *General community.*—Swabs were collected from persons having no direct contact with cases in order to obtain some indication of the general carrier rate. These have been sub-divided into two groups—one containing swabs collected at the time of the main outbreak (1940–42) and the other comprising those taken during an "interepidemic" period (1946–47).

3. *Cases of meningitis.*—Swabs were collected from 22 cases, in 16 of these within 48 hours of onset. Group I strains were isolated from the cerebrospinal fluid of 13 cases but no growth was obtained from the other 9, 3 of which were of the mild, abortive type. The nasopharyngeal swab from one of these mild cases gave a heavy growth of a Group II strain.

4. *Cases of chronic meningococcal septicaemia.*—Two only were investigated.

The results obtained from these swabs are presented collectively in Table III

TABLE III

THE ISOLATION OF THE MENINGOCOCCUS FROM NASO-PHARYNGEAL SWABS

Group	Number examined	Number positive	Group I	Group II
Close contacts (1940–41)	145	21 (14%)	6 (4%)	15 (10%)
General community (epidemic period 1940–42)	410	75 (18%)	11 (3%)	64 (15%)
General community (non-epidemic period 1946–47)	120	8 (7%)	1 (1%)	7 (6%)
Cases of meningitis	22	6 (27%)	4 (18%)	2 (9%)
Cases of chronic septicaemia	2	1	0	1*

*A few colonies of a Group II meningococcus were isolated from the nasopharyngeal swab some 21 days after the onset of symptoms; a Group I strain was isolated by blood-culture.

These results provide little bacteriological evidence to support the "catarrhal" theory. Meningococci were isolated from the nasopharynx of only 6 of 22 cases and in the majority of these the organisms were not numerous; the 1940–42 outbreak was caused almost entirely by Group I strains, but during this period the majority of carriers harboured Group II strains; there was no significant difference in the carrier rates of Group I strains given by the close contacts (4 per cent) and the general community (3 per cent), in both the rates were low; few of the cases or carriers gave a definite history of sore throat or catarrh.

The meningococcus is transmitted by droplet infection, and therefore in all persons developing meningitis these organisms must have been present in the nasopharynx during the initial stages of the infection. Their subsequent isolation from this site is dependent not only on the interval between implantation of the organisms and the collection of the swab but also on the nature of the local reaction. If a local infection develops it is reasonable to expect (1) the isolation of meningococci from the nasopharyngeal swabs in many early cases—not infrequently in practically pure culture, and (2) a relatively high carrier rate of the infecting type of

organism among close contacts, as an interchange of the upper respiratory flora takes place readily among occupants of overcrowded and badly ventilated rooms. Such results have rarely been obtained.

It is recognized that a small number of carriers are of the "persistent" type and that the persistence of the carrier state is associated with some pharyngeal abnormality (Cleminson, 1918; Embleton and Steven, 1919). During this investigation persistent carriers were found to have some abnormality, such as enlarged adenoids, and the majority harboured Group II strains often in relatively pure culture. These organisms were usually associated with a chronic form of local infection, but they were of little epidemiological significance as the main outbreak was caused by Group I strains; they were readily cleared by a course of sulphonamides (Fairbrother, 1940).

The failure to detect the homologous meningococcus in swabs from the two chronic septicaemia cases is not surprising. Logan (1946), in an excellent review of meningococcal septicaemia, points out that the pathogenesis of the disease is uncertain; there is no bacteriological proof that the portal of entry of the organisms is the same in meningococcaemia as in meningitis, although this is probable, while a persistent focus of infection has seldom been found. In the two cases under review, the swabs were collected when the disease was well established and the negative results indicate only that the focus of the existing infection was not the nasopharynx.

Pathogenesis

The path of the meningococcus from the nasopharynx to the cerebrospinal fluid has not been conclusively determined, and in consequence it has been the subject of much speculation. Two main views have been formulated; the first accepts the direct extension of the meningococcus from the pharynx to the meninges, the second postulates that the organisms are transmitted by way of the blood stream.

The theory of direct extension was advocated during the 1914–18 outbreak but convincing proof was not produced. There has consequently been increasing support for the "haematogenous" theory, which is now generally accepted (Brinton, 1941; War Office Memorandum, 1942; Strong, 1943; Banks, 1947). It must, however, be appreciated that the available evidence to support this view is inconclusive and merits examination.

The main arguments advanced in favour of the "haematogenous" route are: (1) the development of a so-called premeningitic stage with involvement of the blood stream; (2) the rare isolation of the organism by blood-culture during the premeningitic phase while the cerebrospinal fluid was normal; (3) the isolation of the meningococcus by blood culture from early cases of meningitis; and (4) the occurrence of a septicaemic form of meningococcal infection in which there is no involvement of the meninges—these infections may occasionally be of a fulminating character.

It has already been pointed out that a satisfactory explanation has not been produced to indicate how the meningococcus enters the blood stream from the nasopharynx. Considerable doubt has, moreover, been cast on the "catarrhal" theory and there is little evidence to indicate that there is a persistent focus of infection in the nasopharynx from which the organisms pass into the blood. Also a satisfactory explanation of the method or route by which the meningococcus passes from the blood to the meninges has not been forthcoming. In this respect, it is interesting to note that there is a definite blood–central nervous system barrier; the passage of substances through the choroid plexus or other vessels into the cerebrospinal fluid is not automatic but is controlled by some form of selective mechanism. It is well known that penicillin injected into the blood does not readily reach the theca; while experimental infection with the poliomyelitis virus is extremely difficult to produce by the intravenous route, although it occurs readily by the intrathecal or intraneural route.

There is no evidence to suggest that the meningococcus has any special affinity for the choroid plexus or other part of the theca. It is indeed accepted that in most cases of meningococcal septicaemia the development of meningitis does not occur even though, in pre-sulphonamide days, the blood infection often persisted for months.

Moreover, a positive blood culture in the early and acute stages of a severe infection cannot be accepted as evidence of primary blood invasion. In such infections as pneumonia and enteric fever a positive blood culture is frequently present in the early stages as a secondary phenomenon, being an overflow of the organisms from the primary focus of the infection. It is, therefore, reasonable to claim that the positive blood culture obtained in many cases of meningococcal meningitis has been a secondary development.

A more serious argument is the rare occurrence of a positive blood culture before the development of meningitis; it must, however, be noted that in several of these cases the primary focus of infection was not established, the presence of a rhinopharyn- ·

gitis being assumed, and/or the presence of meningitis was not excluded by lumbar puncture.

The occurrence of a definite premeningitic clinical stage can also be challenged. Cases of moderate severity tend to give a twenty-four-hour history of general malaise, weakness, headache of varying but usually progressive intensity, and slight neck rigidity. On lumbar puncture a turbid fluid is almost invariably obtained from these cases; this is proof that the invasion of the meninges occurred many hours previously. In fulminating meningitic cases, a turbid fluid is obtained by lumbar puncture even though the history of the infection is short, usually a matter of hours; the possibility of such infections developing in progressive stages (viz., catarrhal and septicaemic) is remote.

There is no specific sign or symptom to indicate involvement of the meninges; the initial symptoms of moderate meningeal infections are often those of an indefinite character, and such cases are not infrequently diagnosed tentatively as influenza. The presence of meningitis can be established only by an examination of the cerebrospinal fluid. The development of a premeningitic phase of the infection cannot therefore be accepted on clinical grounds alone.

In order to test the "haematogenous" theory, blood cultures were prepared as early as possible in the infection from a number of cases, usually at the time of collection of the cerebrospinal fluid and nasopharyngeal swabs. The results are given in Table IV.

TABLE IV

ISOLATION OF THE MENINGOCOCCUS FROM THE BLOOD
AND CEREBROSPINAL FLUID IN CASES OF MENINGITIS

Time of collection	No. examined	No. positive	C.S.F. positive in
Within 24 hours of onset	12	1	12
24–48 hours of onset	8	1	6
Later than 48 hours of onset	5	1	3
Total ..	25	3	21

The meningococcus was isolated from the blood stream in only 3 out of 25 cases (12 per cent). In 20 of the 25 cases the blood was collected within forty-eight hours of the onset of the disease; in all cases a well-established meningitis was present and meningococci were isolated from the cerebrospinal fluid of 21 of the 25.

These results are not consistent with the development of a premeningeal septicaemic stage, but indicate merely the secondary invasion of the blood stream from the primary infection of the meninges. A positive blood culture, obtained when meningitis is present and the cerebrospinal fluid contains large numbers of meningococci, cannot be accepted as evidence of either the passive transfer by the blood stream of the organisms to the subarachnoid space from an indefinite focus of infection in the nasopharynx or the presence of a distinct premeningeal phase.

It is interesting to note that the majority of positive blood-cultures from cases of cerebrospinal meningitis, as distinct from chronic septicaemia, reported in the literature have been obtained when the meningitis was well established.

Discussion

The results of this investigation do not provide evidence, clinical or bacteriological, to support the widely accepted theory that the meningococcus produces a primary pharyngeal catarrh, from which, in relatively few cases, it passes to the meninges by way of the blood stream.

This is not surprising, as evidence advanced to support this theory is far from conclusive. To account for the failure of many cases to provide a history of sore-throat or catarrh, it was suggested that the local pharyngeal infection was often transient and trivial, but bacteriological or clinical proof of this infection was seldom obtained. Moreover there has been no satisfactory explanation of the method by which the organisms enter the blood stream from such a trivial primary lesion or by which they subsequently pass from the blood stream to the meninges.

In view of the fundamental weaknesses of the haematogenous theory it is essential that an alternate method of spread, namely by direct extension, should be carefully examined. This theory received much support during the 1914–18 outbreak, when the following routes were suggested: (1) by the perineural lymphatics of the olfactory nerves through the cribriform plate of the ethmoid; (2) through the sphenoidal sinuses; (3) via the middle ear. Convincing evidence to support any of these routes was not produced, and this theory consequently became neglected.

In 1929, however, Clark produced conclusive proof that material could pass directly to the brain from the nasal passages. Investigating the development of nervous complications following vaccination, Clark showed that a solution of potassium

ferrocyanide and iron ammonium citrate reached the subarachnoid spaces of the brain of rabbits within one hour of being dropped into their nasal cavities. The route of spread was not by the lymphatics but by the perineural sheaths of the olfactory nerves, which provide continuity between the subarachnoid space and the olfactory sensory epithelium; in these spaces a centripetal flow was postulated. It was also found that some of the solution entered the blood capillaries. On treatment of the tissues, the deposit of Prussian blue in the nasal mucosa was found to be considerably more abundant in the olfactory area, which is innervated by the olfactory nerves, than in the "respiratory" portion which is not reached by the olfactory nerves. Clark considered that a similar sequence of events, apart from variations in degree of absorption from the mucosa, was probable in man, and suggested that these findings had an important bearing on the passage of infective material from the nasal cavities to the brain.

It is also interesting to note that, during experimental work on poliomyelitis, Faber (1933) demonstrated that the hairs of the olfactory nerves lie free in the olfactory portion of the nasal mucosa and are covered only by mucus. In spite of this it is important to note that intranasal application of the poliomyelitis virus into the nares of monkeys does not invariably lead to the development of experimental infection even though the monkey is a relatively susceptible animal (Fairbrother and Hurst, 1930). The poliomyelitis virus passes to the central nervous sytem along the axons of the olfactory nerve. This is a different route from that described above, but the area of nasal mucosa at which the neural tract is entered is probably the same in both cases.

There is thus reliable evidence of definite but complex communications between the nasal cavities and the central nervous system; these are situated in the olfactory portion of the nasal mucosa and occupy a relatively small area on the septal and lateral walls of the superior nasal meatus. Passage of infective material along the olfactory tract is not, however, readily accomplished.

Applying these observations to the pathogenesis of meningococcal meningitis, the following hypothesis is postulated.

The meningococcus is widely disseminated by droplet infection and gains entrance to the upper respiratory tract by the nostrils and mouth. The subsequent sequence of events is dependent on many factors, particularly important being the virulence and the number of the organisms, their implantation on the olfactory portion of the nasal mucosa, and the subsequent overpowering of the local defence mechanism.

In the majority of individuals these requirements are not fulfilled; the meningococcus becomes a transient member of the pharyngeal flora, probably as a commensal, and the individual becomes a temporary carrier. Under circumstances particularly favourable to the meningococcus, the local defence barrier is overcome and the organisms may, by direct extension, reach either the theca or possibly the blood capillaries; in the latter event the septicaemic type of infection might result.

Many factors are concerned with the tendency for outbreaks to appear under war-time conditions. During the war 1939–45 opportunities for the dissemination of the meningococcus were greatly increased as a result of overcrowding and poor ventilation, which tended to be accentuated by black-out precautions, while in certain groups, such as new recruits, general resistance was probably lowered by fatigue and exposure, especially during the winter. It is also interesting to note that Group I strains were responsible for the main outbreak and there is much indirect evidence to indicate that these strains are more virulent than Group II.

A possible explanation for occurrence of sporadic Group II infections, found mainly in infants and children, during inter-epidemic periods is the close contact of infants with adults, in particular mothers and nurses, and the consequent greater risk of frequent and large dosage with these strains. Adults may possess some form of specific resistance to the meningococcus, but there is no convincing evidence that the development of specific immunity plays a major role in the defence against meningococcal infections. It is, however, possible that the local defence mechanism is less efficient in infants than in adults, and is more readily overpowered by the relatively avirulent Group II strains.

Summary

The results of this investigation do not support the theory that meningococcal infections occur in three successive stages—catarrhal, septicaemic, and meningitic. Support is given to the theory of direct spread of infection from the nasopharynx to the theca by the perineural sheaths of the olfactory nerves.

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RETICULOCYTES AND THEIR HUMORAL REGULATION

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The reticulocytes are generally accepted to be young red blood cells. Whenever red cell formation in the bone marrow is active the proportion of reticulocytes in the blood increases; hence the importance of the reticulocyte count in haematology. Nevertheless, there have been few studies dealing with purely physiological and chemical aspects. A study of the ripening of reticulocytes into mature non-reticulated red cells has, however, been carried out in our laboratory during the last five or six years. The present article reviews the results obtained.

Experimental Procedure

Rabbits were used as experimental animals. In order to obtain reasonable accuracy in counting their reticulocytes, the animals were brought into a chronic anaemic state by the daily withdrawal of from 30 to 50 ml. of blood. At the end of one week the haemoglobin percentage thus fell to 40 or 50 and the red cell count to 2 or 3 million per c.mm. of blood, with 20 or 30 per cent reticulocytes. This state could be maintained for a month or more before the animals died. The blood of such animals was used in the tests (Plum, 1942a).

Blood was centrifuged and the red cells washed in saline. They were then re-suspended in saline, or in plasma or saline to which had been added the substance whose action on the reticulocytes was to be studied. The suspensions were kept in small test-tubes in a water bath at 40° C. and gently and constantly moved. Samples for reticulocyte counts were taken at regular intervals.

Reticulocytes suspended in saline alone disappeared slowly, but the rate of disappearance could be accelerated considerably by adding commercial liver extract to the saline in which the cells were suspended (Fig. 1). The youngest and most unripe types of reticulocytes disappeared first, and as there was apparently no haemolysis this must have been due to their maturation into "adult" red cells. This process seemed

to be accelerated by some substance found in liver extracts.

The ripening process followed the monomolecular equation well known in physical chemistry and biochemistry:

$$k = \frac{1}{t}\log\frac{a}{a-x}$$

where t is the time of incubation, a the number of reticulocytes at the beginning of the experiment, and x the number of reticulocytes that disappear during the time t; k is the "monomolecular constant." This formula only indicates that the same percentage of reticulocytes is ripened in the same interval of time regardless of the number of reticulocytes. The constant, however, gives a very convenient measure for the rate of ripening; the higher the constant, the faster the rate, and vice versa ; k increases with rising temperature.

Experiments with varying concentrations of liver extract have shown that the constant in the ripening
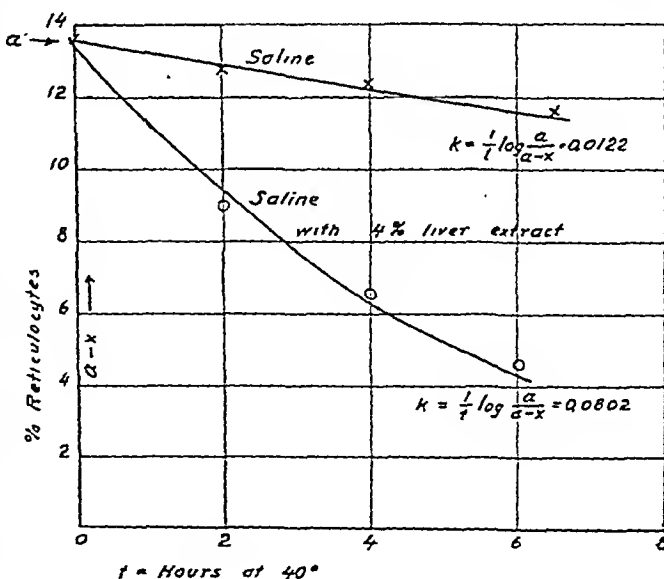
FIG 1.

$$k = \frac{1}{t} \log \frac{a}{a-x}$$
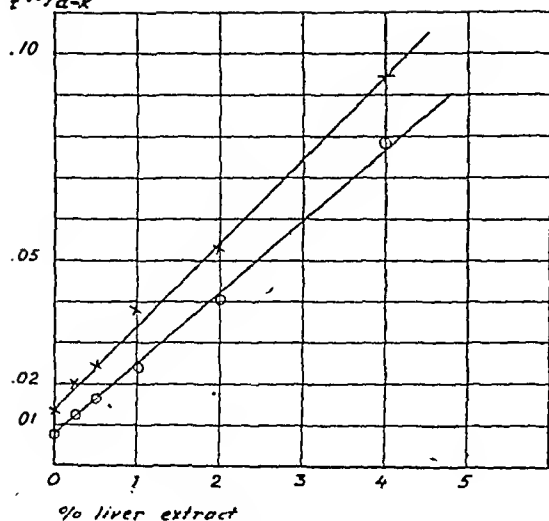
FIG. 2

experiments is directly proportional to the concentration of liver extract (Fig. 2).

Chemical Nature of Reticulocyte-ripening Principle

The reticulocyte-ripening principle in liver extract is thermolabile; it loses its activity after heating for five minutes on a boiling water bath. Thus it cannot be identical with the thermostable principle acting against pernicious anaemia. It has been shown, moreover, that the reticulocyte-ripening principle in liver consists of a thermolabile fraction which can be absorbed to floridine and a thermostable fraction that cannot be absorbed (Jacobsen and Plum, 1942). The latter fraction has little effect by itself, but activates the effect of the thermolabile component. The thermostable fraction can be extracted from liver extract with butanol and isolated from the butanol extract. It has been

TABLE I

Reticulocyte ripening principle
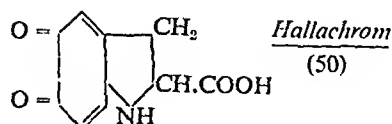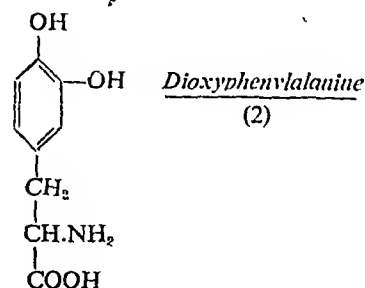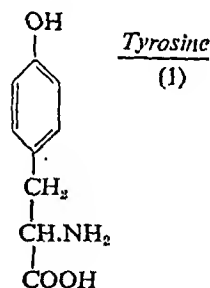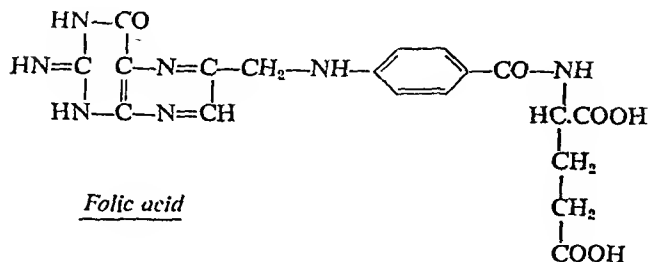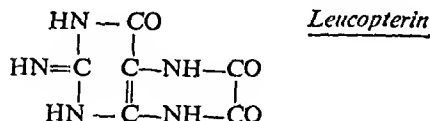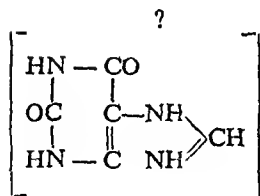
found to be identical with l-tyrosine. Synthetically prepared tyrosine shows exactly the same effect in combination with the thermolabile fraction.

The effect of tyrosine seems to be specific. None of the thirteen most common amino acids, including phenylalanine, has any activating effect on reticulocyte ripening, but compounds similar to tyrosine, such as adrenaline and tyramine, have some effect (Jacobsen and Plum, 1942). Later investigations have shown that certain oxygenated products of tyrosine, dioxyphenylalanine and hallachrom, have a much stronger activating effect, that of hallachrom being 50 to 100 times stronger than that of tyrosine (Gad and others, 1944). As there are enzymes present in red cells capable of converting tyrosine into hallachrom it is possible that the activating effect is not due to tyrosine, but to hallachrom derived from it.

The thermolabile reticulocyte-ripening fraction has not been isolated from liver. Systematic investigations on various organs have shown, however, that stomach tissue (Plum, 1944a), duodenum, and the upper part of the small intestine contain considerable amounts of the thermolabile fraction (Bohn, 1946). Extracts from these tissues have little ripening activity by themselves, but marked ripening effects, even greater than that of liver extracts, have been found after the addition of tyrosine, dioxyphenylalanine, or hallachrom. The ripening fraction in gastric tissue is extremely labile; however, a rather powerful preparation

containing 75 to 80 per cent xanthine has been isolated from it (Jacobsen, 1944). Synthetically prepared xanthine in combination with tyrosine has a marked effect, but it does not seem likely that the effect of the gastric extract can be explained by its content of xanthine. Probably a tautomeric isomer is responsible. Some chemical analogues of xanthine have been tested. Guanine and caffeine amongst others are ineffective. Recently both leucopterin and folic acid have been shown to be more potent than xanthine, although with qualitative differences. Xanthine is only effective in the presence of tyrosine and is thermolabile. Leucopterin and folic acid have, however, an effect even without tyrosine, but are activated 100 per cent or more after its addition. The potency of neither of these compounds is affected by boiling.

The chemical problem has not been solved. Several experiments indicate that the thermolabile fraction consists of more than one substance. The fact that substances which have recently been shown to have an effect on haemopoiesis *in vivo* may accelerate ripening of reticulocytes *in vitro* is, nevertheless, of great interest.

CONTENT OF RETICULOCYTE-RIPENING FACTORS IN ORGANS AND PLASMA

As has already been stated, reticulocyte-ripening principles may be found in extracts from several organs of the body. Calculated per gramme of tissue, the greatest concentration is found in plasma, and slightly less in bone marrow, liver, stomach, and spleen (Plum, 1944a). Extracts of all these organs, but not plasma, may be activated by the addition of tyrosine (Fig. 3). The stomach and the upper intestinal tract contain the highest concentration of the thermolabile fraction. Large amounts are found in the duodenum and in the upper part of jejunum, apparently following the distribution of Brunner's glands. The results of chemical investigations on stomach extracts have already been mentioned. In Fig. 3 is shown the distribution in swine; similar relative distributions are found in the organs of oxen, rats, rabbits, and guinea-pigs.

FIG. 3

□ *without tyrosine*
▨ *with tyrosine*

B
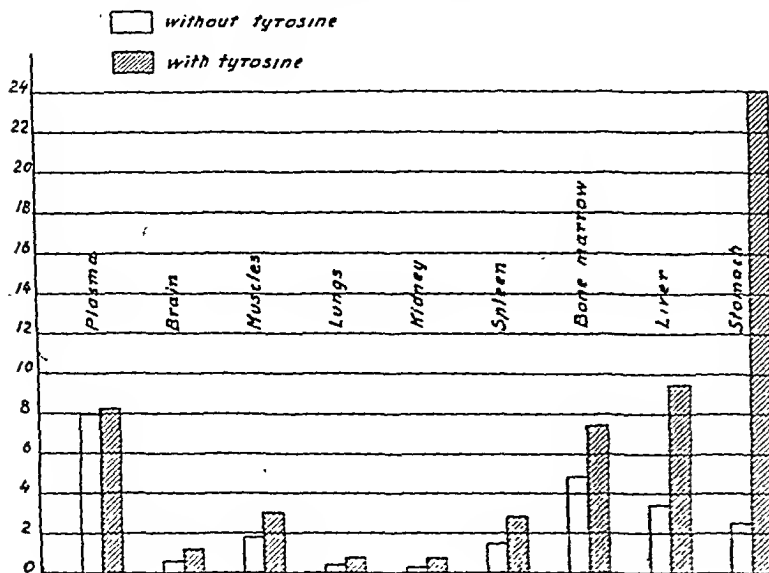
Unlike liver extracts the reticulocyte-ripening principle in plasma cannot be activated by tyrosine, and plasma is not able to activate the thermolabile fraction further as it ought to if it contained tyrosine or tyrosine-like substances. However, when plasma is treated with acetone, the ripening principle is transformed into a state in which it can be activated by tyrosine. This shows that there is no great difference between the ripening principles in plasma and liver; the linkage between thermolabile and thermostable fractions in plasma must be a strong one.
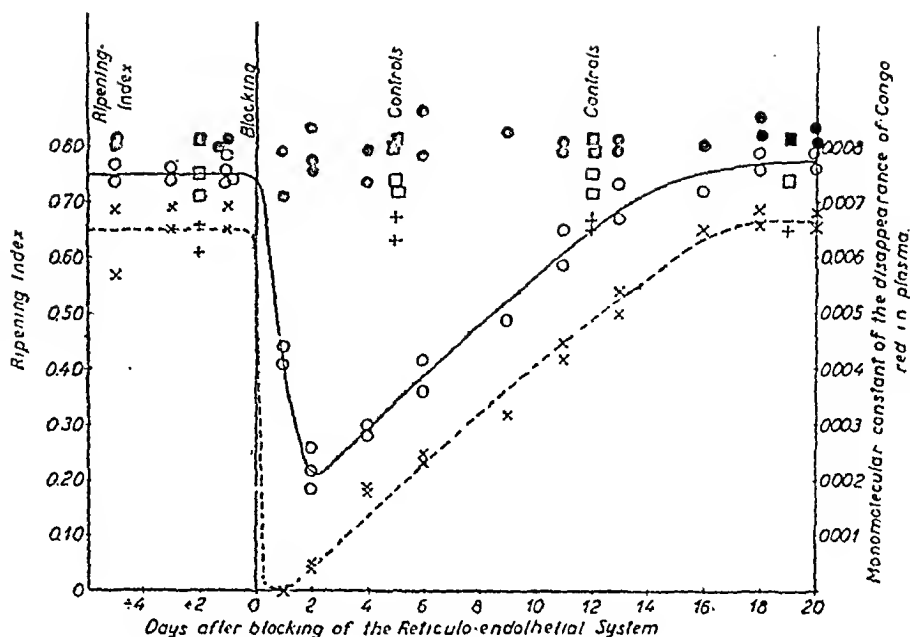
FIG. 4

ROLE OF THE RETICULO-ENDOTHELIAL SYSTEM

Experiments with rabbits have shown that the action of the thermostable component is influenced by the activity of the reticulo-endothelial system. The content of ripening substances in plasma has been determined with and without the addition of tyrosine. After a control period the reticulo-endothelial system was blocked by intravenous injection of 10 ml. 1 per cent trypan blue combined with splenectomy. The activity of the reticulo-endothelial system, as tested for by the disappearance of congo red, rapidly drops to zero and recovers slowly, becoming normal again by the sixteenth to eighteenth day. Fig. 4 demonstrates that the amount of ripening principle in plasma closely follows the activity of the reticulo-endothelial system; after addition of tyrosine, however, there is no difference from the normal. Blocking has thus no influence upon the thermolabile component.

These findings make it seem probable that tyrosine (or a derivative of, or an oxygenated product of, tyrosine) and the thermolabile substance are linked in the reticulo-endothelial system and together form the reticulocyte-ripening principle found in plasma (Jacobsen and Plum, 1943a).

Content of Reticulocyte-ripening Principles in the Plasma of Various Animals and Man

As the content of the ripening factors in organs varies with that in plasma, most of the investigations
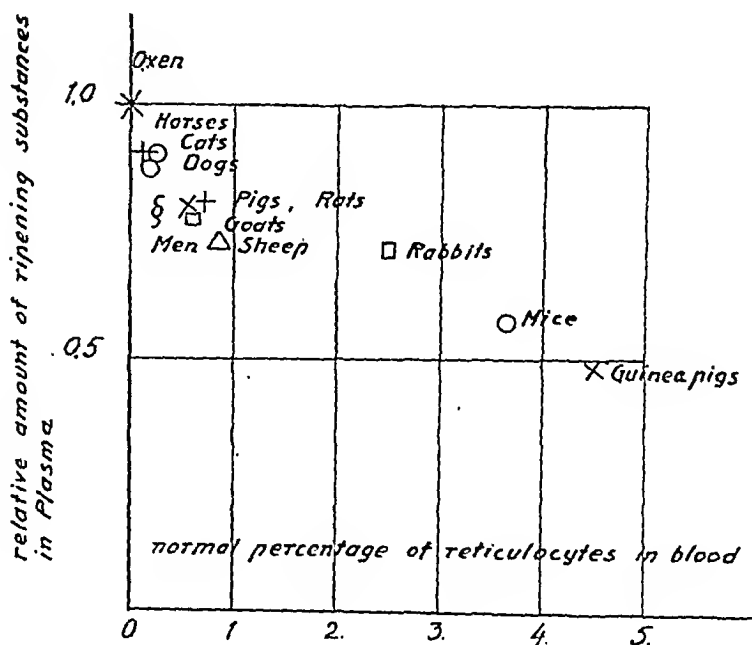
FIG. 5

have been carried out on the plasma of various animals. The amount of reticulocyte-ripening factor in plasma varies in different animals (Plum 1942b, 1943). Reptiles and birds have practically none. In mammals there is considerable variation (Fig. 5). The plasma of animals whose blood normally contains few or no reticulocytes (for example, oxen) seems to contain the highest content of ripening substances, whilst that of species whose blood normally has a relatively high percentage of reticulocytes (e.g., guinea-pigs) has the lowest content, in the case of guinea-pigs half that of oxen.

The content of ripening substances in the plasma of normal adults also seems to vary inversely with the reticulocyte count. In certain physiological and pathological states, however, this relationship may not hold (Table II).

TABLE II

|	Reticulocytes	Ripening substances
Early life	+++	+++
Anaemia due to loss of ery-throcytes	++	+
Iron deficiency	+	+
Benzol poisoning ..	+	+
Gastrectomy or duodenec-tomy	(÷)	—
Pernicious anaemia ..	?	—
Hypothyroidism		—
Hyperthyroidism	0	—
Hypophysectomy ..		—
Menstruation	(+)	(÷)
Jaundice, obstructive and haemolytic	(÷)	(÷)

In foetal life and in infancy until the blood picture becomes normal, the increased numbers of reticulocytes are associated with a considerably increased amount of ripening principles. In newborn rats or rabbits with 80 or 100 per cent of their red cells in the form of reticulocytes, about double the normal concentration of ripening substances may be found. Both reticulocyte percentage and content of ripening substances decrease gradually and reach normal values at the same age (Plum, 1943). A probable explanation of the occurrence of high reticulocyte percentages and high concentrations of ripening substances, for which there is experimental support, is that the immature blood corpuscles in the early ages react more slowly to humoral stimulation (Jacobsen and Plum, 1943b).

The reticulocytosis associated with increased blood formation, as seen in chronic post-haemorrhagic anaemia in rabbits, is always combined with an increase in ripening substances (Plum, 1943). In this case the response of the reticulocytes to a concentration of ripening substances does not change, but the quantity of ripening substances in the plasma increases. This increase may cause the reticulocytes in the peripheral blood stream of anaemic rabbits to ripen about 20 or 30 per cent more rapidly than in normal animals. The same phenomenon is seen when anaemia is caused by some haemolytic poison such as phenylhydrazine (Plum, 1944b).

Similarly, in iron-deficiency anaemia of dietary origin reticulocytosis is accompanied by an increased concentration of reticulocyte-ripening substances (Plum, 1944b). On treatment with iron, both anaemia and reticulocytosis disappear and the content of reticulocyte-ripening substances in the plasma returns to normal. Anaemia and leucopenia result from the effects of benzol poisoning on the bone marrow. The reticulocyte count may fall or rise slightly, but the content of reticulocyte-ripening substances in the plasma is always increased. During regeneration with increases in leucocytes and reticulocytes the content of ripening substances returns to normal. These experiments suggest that the rise in reticulocyte-ripening substances may be interpreted as one of the measures by which the body attempts to compensate for anaemia.

Occasionally an increased number of reticulocytes is associated with reduced amounts of reticulocyte-ripening principles in the plasma, as in rats after gastrectomy (unpublished observations) and in swine in which the duodenum had been either isolated or excised causing a 20 per cent decrease in activity (Bohn and others, 1945).

In untreated pernicious anaemia a decrease up to 40 per cent has been observed. The addition of tyrosine has in all cases raised the concentration of ripening substances up to the normal level. This decrease is thus not due to a decreased concentration of the thermolabile component, as might have been expected from the known high concentration of the thermolabile fraction in the gastric tissue of the normal subject and the characteristic gastric atrophy in pernicious anaemia.

Both in spontaneous hypo- and hyperthyroidism in man and in the experimental animal there is a decreased content of ripening substances in the plasma, which may be corrected by the addition of tyrosine. The decrease after hypophysectomy may result from decrease in the function of the thyroid gland.

During menstruation a slight increase has been observed starting on the first day.

Obstructive jaundice in patients and in animals may be associated with a slight increase in ripening substances.

Conclusion

Assay of the concentration of reticulocyte-ripening substances in the plasma is of more scientific than practical interest. It has no diagnostic value. The plasma of from one hundred to one hundred and fifty patients with various diseases and that of about fifty normal persons have been tested. A definite decrease in the content of ripening substances in the plasma has been found in untreated pernicious anaemia, in Graves's disease, in some patients with gastric diseases, and in cachectic patients suffering from cancer (Plum, R., 1947). In all cases this decrease has been due to lack of the thermostable fraction and could be rectified by the addition of tyrosine. In some patients with jaundice the content of the ripening substances has been increased. In all other diseases normal levels have been found.

Even a slight reticulocytosis has been generally considered a sign of increased erythropoiesis. The work now described indicates that this may not always be the case. The reticulocyte percentage in peripheral blood depends upon the output of reticulocytes from the bone marrow, controlled by factors still unknown, and the rate of ripening in the blood stream, the latter regulated by the ripening principles. Thus with a constant production the numbers of reticulocytes in the circulating blood can increase if the concentration of ripening substances in plasma decreases, and vice versa. Slight reticulocytosis cannot, therefore, be regarded as a sign of increased or altered blood production unless it is certain that the concentration of ripening substances has not altered.

It is difficult to make any definite statement with regard to the physiological role of the ripening substances, particularly as it is doubtful what reticulocytes really are. It is thought that under normal conditions not all the red cells released from the bone marrow are in the form of reticulocytes. As the capacity of the reticulocytes to transport oxygen is as good as that of adult cells, it is doubtful whether their maturation into adult corpuscles is of any great physiological significance. The as yet unpublished observations of my collaborator, Dr. C. M. Plum, who has done a major part of the work described in this paper, seem to show that a similar mechanism may operate in the formation of red cells from normoblasts. If this is true, the ripening of reticulocytes can be regarded as a special case or model of the course of erythropoiesis as a whole. The demonstration of a humoral regulation of blood-cell formation may be expected to throw new light on many of the hitherto unsolved problems in erythropoiesis.

Summary

A principle capable of accelerating the ripening of reticulocytes *in vitro* can be demonstrated in plasma and in various tissues of the body.

This principle consists of at least two fractions: a thermostable one, identified as tyrosine or tyrosine derivatives, and a thermolabile one. Xanthine, leucopterin, and folic acid are able to act as the thermolabile factor. The greatest concentration of the thermolabile fraction is found in the stomach and duodenum. The thermolabile and thermostable fractions linked together by the activity of the reticulo-endothelial system form the principle found in plasma.

An increased amount of ripening principle may be found in the plasma in some cases of increased erythropoiesis; in decreased erythropoiesis a lower content than normal may be encountered. It seems as if the number of reticulocytes in the blood varies inversely with the amount of ripening principle, at least under normal conditions.

The significance of these findings is discussed.

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THE DISTRIBUTION OF LEUCOCYTES ON
THE COUNTING CHAMBER

BY

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It is almost a statistical truism that the number of cells on each square of a haemocytometer is distributed in a Poisson series, but in fact it is easy to show that the distribution departs considerably from the theoretical expectation. The reason, perhaps, is that the experimental work confirming this theory was done on the old well type of haemocytometer, whereas the Bürker counting chamber is now universally used, and adds new and less predictable variations to random sampling.

My attention was drawn to this point by a statistically controlled series of leucocyte counts on Bürker counting chambers with the Neubauer ruling. I counted, as is usual, the number of cells on four areas of one square millimetre at the corners of a square with a side of three millimetres, and recorded the four sub-totals separately. Over the whole series, the two squares nearest to the filling point of the chamber gave nearly identical totals 3.5 per cent below the mean value for all four squares, and the two further squares similarly gave nearly identical totals 3.5 per cent above the mean value. The difference was statistically highly significant. It seemed worth while to test the extent of this deviation from the theoretical Poisson distribution, and to see whether it might be a source of error in blood counts.

Methods

The Bürker counting chambers used were by several different manufacturers, but all had some variety of Neubauer ruling. The cover-slips were from 0.4 to 0.5 mm. thick, and their position on the chambers was fixed by reference to permanent marks.

Blood from a finger-prick was diluted 1/10 in a leucocyte pipette with 2 per cent aqueous acetic acid

coloured with 50 mg. of crystal violet per 100 ml. The contents of the pipette were mixed by vigorous shaking for two and a half minutes, half the contents were rejected, and the counting chamber was filled with care to avoid bubbles and over-filling. The chamber stood on a perfectly flat surface until the leucocytes had settled.

Areas of 0.1 sq. mm. on the counting chamber were defined by means of an eye-piece graticule and a ⅙-inch objective, and the mechanical-stage vernier was used to set the fields of observation in rows 1 mm. apart across the length of the chamber. In each row the cells were counted in nine fields with their centres 0.5 mm. apart. In this way the whole filled area of the counting chamber was covered by a grid of observations with its boundaries 1 mm. from the edges of the cover-slip and the troughs.

The numbers of leucocytes observed in the grid of areas was entered into a table of equidistant columns along the length of the chamber, and of equidistant rows running across the chamber. The totals for the nine columns over a large series of separate counts did not differ significantly, that is, the side-to-side variation in the leucocyte distribution did not differ from expectation. It was, therefore, possible to measure lengthwise variation in the leucocyte distribution simply by reference to the "row" totals, which were large enough to prevent the Poisson distribution from interfering with the analysis of variance.

With each chamber and length of cover-slip the counts were repeated until 500 to 800 cells had been counted in each position along the length of the chamber. The "row" totals in each count formed the basis of the statistical analysis. In all, some 150,000 leucocytes were counted.

Single-cell Counting Chambers

A series of leucocyte counts was made on single-cell counting chambers fitted with cover-slips 16 mm. long in the longitudinal axis of the chamber. In
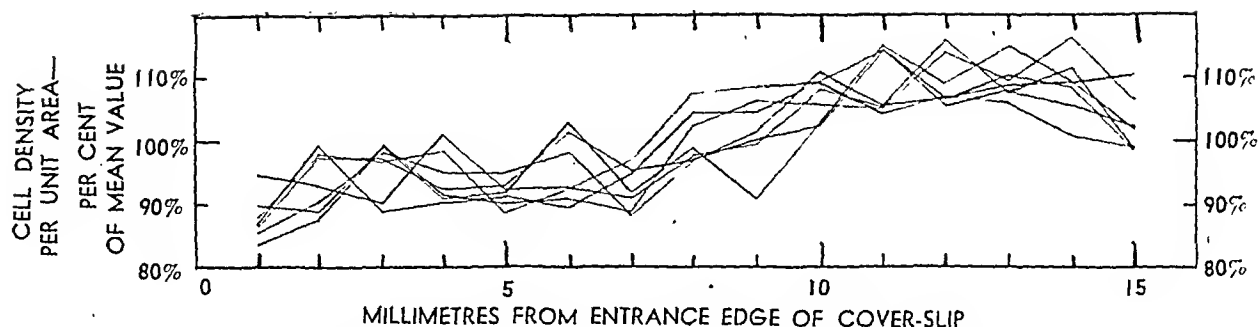
FIG. 1.—The leucocyte distribution on seven single-cell counting chambers. About ten counts were done on each chamber.

Fig. 1 each "curve" represents the total of nine or ten separate counts on seven counting chambers, each with its own cover-slip. Considerable random variation is to be expected in cell counts of the order of 500, but in spite of this a very definite and constant trend in the leucocyte distribution is evident. The statistical analysis of these figures is summarized in the Table as an example of the methods used throughout the investigation.

TABLE

THE ANALYSIS OF VARIANCE OF THE LEUCOCYTE TOTAL IN FIFTEEN POSITIONS ON THE COUNTING CHAMBER IN SIXTY-NINE BLOOD COUNTS

	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Between bloods	68	226,164.87	3,325.95	48.0***
Between places	14	18,424.61	1,316.04	19.0***
Residue ..	952	65,893.69	69.216	
Total ..	1,034	310,483.17		
Between places	14	18,424.61	1,316.04	19.0***
Linear (ξ_1)	1	13,723.40		
Remainder	13	4,701.21	361.63	5.22***
Quadratic (ξ_2)	1	273.23		
Remainder	12	4,427.98	369.00	5.33***
Cubic (ξ_3)	1	2,049.92		
Remainder	11	2,378.06	216.19	3.13***
Quartic (ξ_4)	1	1,344.89		
Remainder	10	1,033.17	103.32	1.49
Quintic (ξ_5)	1	423.30		
Remainder	9	609.87	67.76	

The analysis of variance is first divided between "bloods"—due to the different leucocyte contents of the 69 specimens of blood used—and between "places"—representing the varying density of leucocyte distribution along the length of the counting chamber. The residual variance is a measure of experimental error, and is made up mostly of the component due to random sampling from a basically Poisson distribution. (The mean "row" total was 57.939.)

The significance of the difference between places is beyond question, so we next determine whether the variation in leucocyte density along the counting chamber can be represented by a straight line, or whether a more complex curve is necessary. This point is tested by fitting successive orthogonal polynomials (Fisher and Yates, 1943). The second half of the analysis in the Table shows how the calculation of each successive term removes a part of the sum of squares with a degree of freedom, until the remaining variance is not significantly greater than the residual variance. An alternative comparison is between the mean square from each term of the polynomials and the corresponding remainder mean square. This method gives the following values for the variance ratios:

ξ_1 38.0*** ξ_2 subnormal ξ_3 9.48* ξ_4 13.01** ξ_5 6.25*.

All the terms except the quadratic are significant.

The conclusion is that the variation in leucocyte distribution along the counting chamber cannot be represented by a straight line, but demands a quartic polynomial. This is a curve with a maximum towards either end of the cover-slip, as is shown in the subsequent figures.

It has often been surmised that the lines of the ruled area disturb the cell distribution on the counting chamber, but no significant effect was demonstrated in these observations. Fig. 2 contrasts the leucocyte distribution when the ruled area was under the centre of the cover-slip and when it was near to either end. The difference between the three curves is no greater than would be expected from random variation.

The total length of counting-chamber under the cover-slip has a considerable effect on the shape of the leucocyte distribution curve (Fig. 3), but all the curves have one important common property—that the leucocyte density reaches its mean value half-way along the cover-slip. If, therefore, the ruled area does not lie exactly under the centre of the cover-slip, the count will tend to be either too high or too low. The curves further show that the longer the cover-slip the less is the error from the ruled area not being
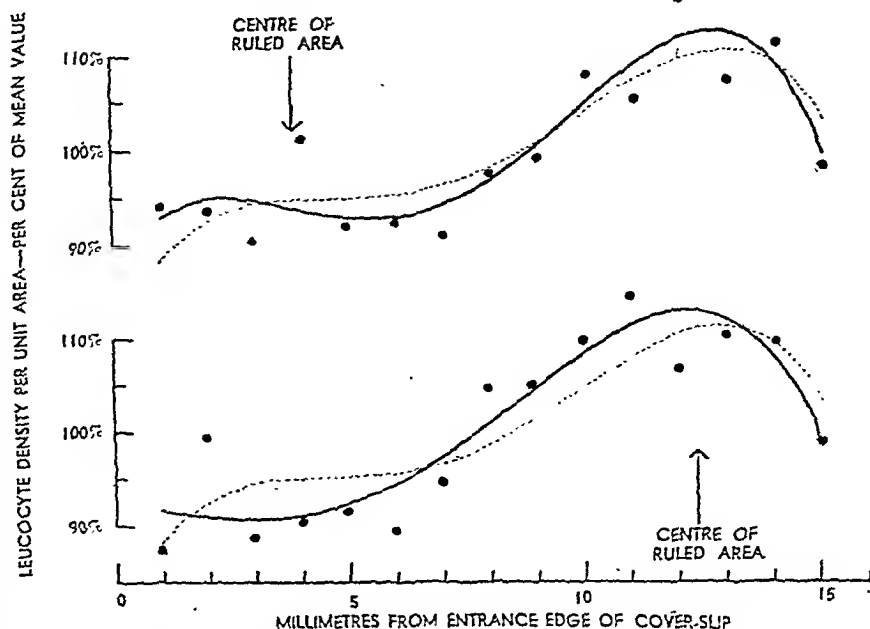
Fig. 2.—The leucocyte distribution on single-cell counting chambers with the ruled area under the centre of the cover-slip (broken line) or towards either end (continuous lines). The curves are the best-fitting quartics; the dots show the observed values relating to the continuous lines.
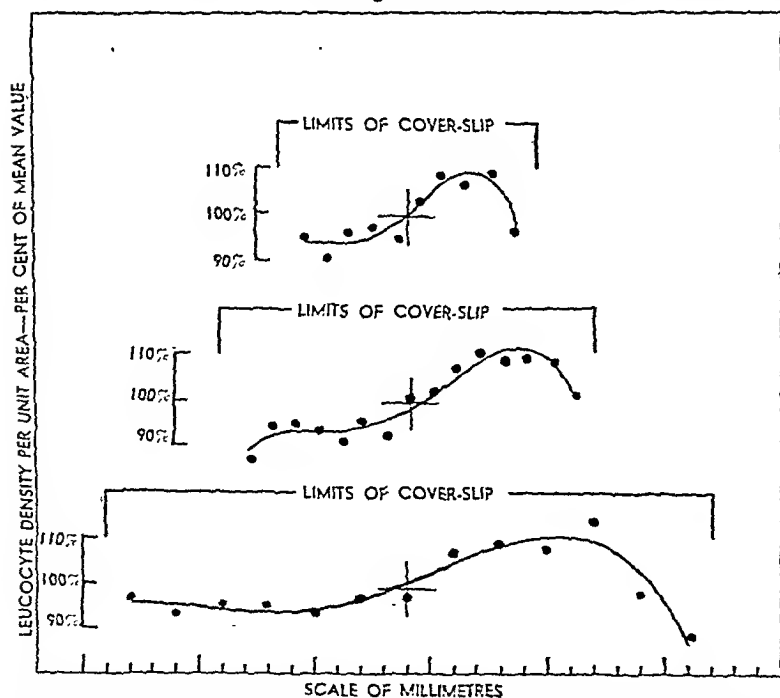
Fig. 3.—The leucocyte distribution on single-cell counting chambers with cover-slip of different lengths. The dots mark the observed values; the curves are the best-fitting quartics. The crosses mark the centre of the cover-slip and the mean value.

central. If the ruled area lies 1 mm. away from the centre of the cover-slip, the error introduced is 4 per cent if the cover-slip is 11 mm. long, 3 per cent if it is 16 mm. long, and 2 per cent if it is 26 mm. long.

Double-cell Counting Chambers

The leucocyte-distribution on double-cell counting chambers (with a central trough and two ruled areas) varies in a very similar fashion (Fig. 4). Again the rule holds that the leucocyte density reaches its mean half-way along the cover-slip. The cover-slip should therefore be so placed that its edge and the edge of the central trough are equidistant from the centre of the ruled area. This is easily ensured by a suitable
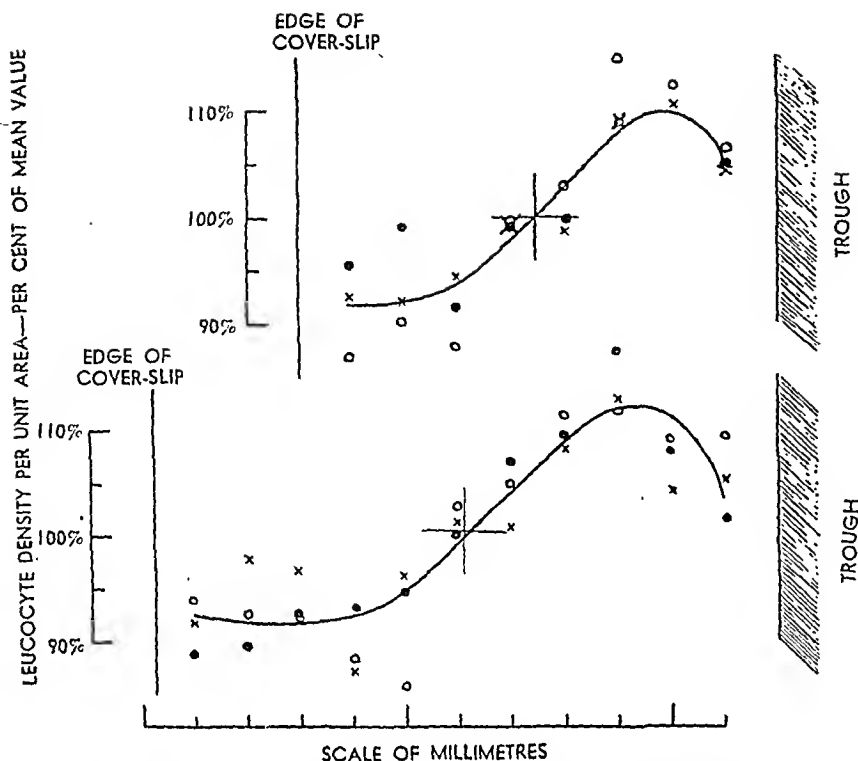
Fig. 4.—The leucocyte distribution on one side of double-cell counting chambers. The observed values from twelve counts on each of three counting-chambers with two cover-slips lengths are marked; the curves are the best-fitting quartics to the whole series. The crosses mark the mean value and the centre of the filled area of the cell.

Reasons for the Effect

It is very unlikely that this variation in the leucocyte density on the counting chamber is due to bending of the standard cover-slips under capillary tension. A bending of this sort should produce a symmetrical distortion of the leucocyte distribution, rather than the asymmetrical distortion demonstrated, and different cover-slips would not produce such very similar effects.

The possibility that the uneven leucocyte distribution arose in the capillary stem of the pipette was also excluded. A 1/10 dilution of blood in leucocyte-counting fluid was made in small tubes containing glass beads, and after thorough mixing a drop of the fluid was transferred to a counting chamber with a platinum loop. It is not possible by this technique to fill the counting chamber with real accuracy, but the leucocyte distribution on the counting chamber agreed reasonably well with that obtained by the ordinary method (Fig. 5).

The true explanation of the uneven leucocyte distribution on the counting chamber seems to be the drift of the leucocytes along the chamber. When

mark when only one side of the counting chamber is in use, but if both sides are used simultaneously the cover-slip itself must be of the proper size.

The ruled area should be as far as is reasonably practicable from the central trough (5 mm. is a suitable distance), for the longer the area under the cover-slip the less is the slope of the leucocyte-distribution curve. Thus if the area covered is 9 mm. long, a displacement of 1 mm. of the cover-slip from "true centre" will lead to an error of 4.5 per cent, whilst if the area is 12 mm. long a displacement of 1 mm. will lead to an error of only 2 per cent.

Much greater errors may be introduced if no attempt is made to position the cover-slip. Some counting chambers have the centre of the ruled area only 3 mm. from the central trough; if 12 mm. of the cell in such a chamber were filled the leucocyte count would be nearly 12 per cent too high.

Fig. 5.—The leucocyte distribution on a counting chamber filled from a leucocyte pipette (broken line) and by the "tube and loop" technique (continuous line). The curves are the best-fitting quartics; the dots mark the observed values relating to the continuous line.

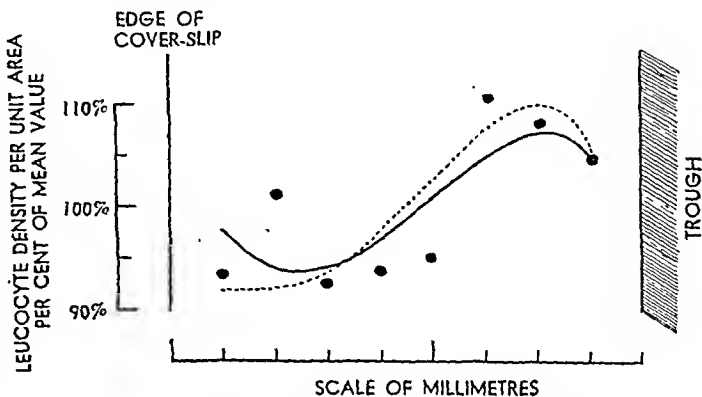the chamber is filled, the leucocytes, having a greater density than the diluting fluid, will continue to flow forward after the fluid has stopped. The higher above the surface of the counting chamber a cell is when filling is completed, the further along the chamber the cell will float before it settles. Thus the nearer end of the chamber will lose leucocytes which will concentrate towards the further end.

This initial impetus of the leucocytes must soon be lost, but observation shows that any floating leucocyte is usually moving along the longitudinal axis of the counting chamber. This movement, which is probably engendered by convection currents as the fluid dries, is most marked at the two ends of the cover-slip, and is in the direction of the closer edge. Thus the secondary leucocyte drift tends to remove leucocytes from the middle of the cover-slip and to deposit them towards either end, so that the leucocyte-distribution curve has the two maxima shown above.

Floating leucocytes very rarely show any appreciable lateral drift, so that in any narrow segment across the chamber the leucocyte distribution obeys the Poisson law.

Discussion

It is clear that considerable errors can be introduced into a leucocyte count if account is not taken of the variation in leucocyte density along the counting chamber. The count may be as much as 10 per cent too low if cells are counted in an area between the "entrance edge" of the cover-slip and the centre of the filled area, or 10 per cent too high if cells are counted beyond the centre. The true leucocyte count is obtained only if cells are counted in areas symmetrically disposed about the centre of the filled area.

The leucocyte distribution curve has a steep gradient in the middle third of the filled area, but the steepness decreases as the length of counting chamber filled increases. With a length of 9 mm. the leucocyte density per unit area changed at the rate of 5 per cent per mm. at the centre of the filled area, whereas with a length of 26 mm. the rate of change was only 2 per cent per mm.

Thus the longer the area of counting chamber filled, the less will be the error from small inaccuracies in placing the cover-slip.

We should therefore make some new rules for blood-counts. With single-cell chambers the cover-slip should cover as much as possible of the chamber, and should be so placed that the ruled area lies exactly under its centre. With double-cell chambers, since both sides will normally be used together, the cover-slip should be of such a length that both

ruled areas lie midway between an edge of the cover-slip and the nearer edge of the central trough. The ruled area should be as far as possible from the central trough, so long as it can still be centred under the cover-slip. Finally, when the cover-slip has been properly placed it is still necessary to remember the steep change in leucocyte density across the ruled area, and to count squares symmetrically disposed about the centre.

It is probably not possible to use the leucocyte-distribution curves obtained above to obtain a precise correction factor when the ruled area is not truly central. The central position of the mean value seems to be constant, but the shape of the curves must be influenced by many factors such as the viscosity of the diluting fluid and the rate of evaporation.

It seems, from a limited number of experiments, that the red-cell distribution on the counting chamber varies in a similar way to the leucocyte distribution. The slope of the curve is less, but the rule holds that the mean value is reached in the centre of the filled area.

Summary

1. The density of leucocytes per unit area on the counting chamber increases progressively from the point of entrance of the fluid along the length of the chamber.

2. The cell distribution in a narrow segment across the chamber obeys the Poisson law.

3. The curve of the leucocyte distribution along the chamber has two maxima, and statistically is adequately represented by a quartic polynomial.

4. The position of the ruled area does not affect the shape of the leucocyte-distribution curve.

5. The mean value for the leucocyte density per unit area is reached at the centre of the filled area of the chamber.

6. The rate of change in the leucocyte density is less the longer the filled area of the counting chamber.

7. The variation in the leucocyte distribution is not due to bending of the cover-slip, nor to any change in the cell distribution in the pipette before the chamber is filled. It appears to be due to the drift of the leucocytes caused by their initial forward impetus and subsequent convection currents.

8. Recommendations are made to counteract errors due to variation in the leucocyte distribution.

9. The distribution of red cells on the counting chamber follows similar laws to the distribution of leucocytes.

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THE COLORIMETRIC DETERMINATION OF GLUCOSE

BY

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Several new descriptions of colorimetric methods for the determination of blood sugar have been described in recent years (for example, Polis and Sortwell, 1946; Somogyi, 1945; Nelson, 1944; Haslewood and Strookman, 1939). These procedures are all based on the use of alkaline copper reagents and on the production of molybdenum blue by the action of cuprous copper on complex molybdic acid solutions. With a view to finding the method or combination of methods which gives the most reliable results, particularly in the very low range of blood sugar, the following combinations of reagents were compared: (*a*) Harding's Schaffer-Hartmann copper reagent with the Folin-Wu phosphomolybdic acid; (*b*) Harding's copper reagent with the Nelson arsenomolybdic acid; (*c*) Somogyi's copper reagent with the Folin-Wu phosphomolybdic acid; and (*d*) Somogyi's copper reagent with the Nelson arsenomolybdic acid.

Much difficulty has occurred during the war years and subsequently in this country with colorimetric methods for blood sugar. Biochemists have repeatedly complained of the rapid fading of the blue colours. This fault has been more manifest because of the recent introduction of photo-electric instruments in which the rapid falling-off of the intensity of the blue colour has been noted by numerous workers. With Duboscq colorimeters the fading would not have been so readily noticed, since the rate of fading in standard and test is very similar. It has frequently been impossible during the last several years to obtain chemicals of Analytic Reagent quality, and this abnormal behaviour has been traced to the use of inferior chemical reagents. The ordinary or technical grade of sodium carbonate, for instance, contains considerable sodium bicarbonate as well as the sodium carbonate. The amount of this inferior reagent specified for the preparation of an alkaline copper solution contains insufficient sodium carbonate to produce the desired properties in the final solution, and an abnormal behaviour consequently results.

Experimental

REAGENTS

Harding's copper reagent.—The modified Harding-Schaffer-Hartmann reagent is described by Haslewood and Strookman (1939). This reagent is identical with that described by Harding and Downs (1933), except for the omission of the potassium iodide. Its preparation is also described by King (1947).

Somogyi's copper reagent.—Prepared according to Somogyi (1945).

Folin and Wu's phosphomolybdic acid.—Prepared according to Folin and Wu (1920).

Nelson's arsenomolybdic acid.—Prepared according to Nelson (1944), King (1947).

Glucose standards.—A stock solution is made by dissolving 0.1 g. anhydrous glucose in saturated benzoic acid solution and making up to 100 ml.; 2, 3, and 5 ml. of the stock solution are diluted to 100 ml. with saturated benzoic acid to give working standards of 0.02, 0.03, and 0.05 mg. per ml. respectively (equivalent to 80, 120, and 200 mg. glucose per 100 ml. of blood, according to the following method).

Isotonic sodium sulphate–copper sulphate solution.—3 g. Na_2SO_4, 10 H_2O, and 0.6 g. $CuSO_4$, 5 H_2O are dissolved in water and made up to 100 ml.

Sodium tungstate.—10 g. per 100 ml.

PROCEDURE

Preparation of blood filtrate.—0.05 ml. of blood is added to 1.85 ml. of the isotonic sodium sulphate-copper sulphate solution in a conical centrifuge tube, and 0.1 ml. of sodium tungstate is added. The mixture is shaken, and the precipitated proteins and copper tungstate are spun down in the centrifuge, or filtered through a small paper.

Estimation of glucose.—1 ml. of the above filtrate and 1 ml. each of the glucose standard solutions are measured into 3/4-in. diameter test tubes; 1 ml. of copper reagent is added and mixed. The test tubes are stoppered with cotton-wool and heated in a boiling water bath for exactly 10 minutes. They are cooled in running water, and 3 ml. of Folin-Wu phosphomolybdic acid, or 1 ml. of Nelson's arsenomolybdic acid, with water to 5 ml. (for Duboscq; 10 ml. for photo-electric) are added. The colours are allowed to develop for 10 minutes and are then compared.

Results

REAGENTS PREPARED FROM "ORDINARY GRADE" CHEMICALS

Comparison of colorimetric methods.—Several dilutions (in addition to the above) were made of the stock standard glucose solution. One ml. samples were treated according to the above procedure and the colours measured in a photo-electric absorptiometer with the Ilford spectrum red filter. The zero adjustment was made with water in the cuvette. The results (Fig. 1) showed that the com-
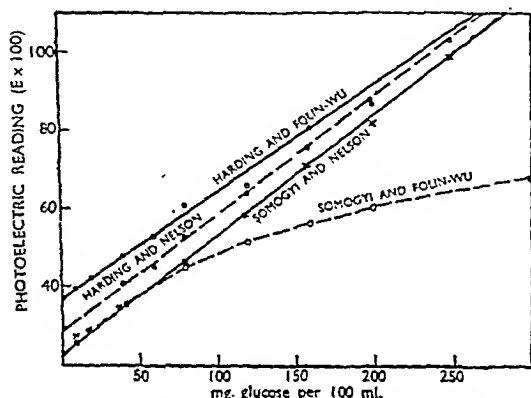

FIG. 1.—Photo-electric readings (E × 100) of glucose solutions estimated by different methods. Chemicals of ordinary quality (not analytical reagent grade) were used in preparing the reagents. Note the high "blank" colours.

binations of Harding with Folin-Wu, Harding with Nelson, and Somogyi with Nelson, furnish procedures capable of giving good conformability with Beer's Law ; but that the Harding–Folin-Wu mixture produced a considerably larger blank than the others. The Somogyi–Folin-Wu combination yielded much less satisfactory figures whose proportionality fell off rapidly with increasing concentrations of glucose.

Stability of colours produced.—Standard solutions containing amounts of glucose equivalent to 60 and 120 mg. glucose per 100 ml. and a water blank were prepared with the Harding copper and Folin-Wu phosphomolybdic acid. Rapid fading was found to occur in the final blue solutions with loss of proportionality (Fig. 2). It will be seen that a considerable amount of reduction occurred in the blank. This was, in fact, visible to the naked eye, there being a greenish precipitate of cuprous oxide when the tube was removed from the water bath.

The reagents used in the above experiments had been prepared when no Analytical Reagent chemicals were available. It had previously been pointed out from this laboratory (King, Haslewood, and Grant, 1936) that impure sodium carbonate cannot be used for the titrimetric method for glucose, and it is apparent now that it is equally dangerous to use it for the colorimetric procedures. It was observed that when the Folin-Wu phosphomolybdic acid was prepared from wartime molybdic acid, a considerable amount of ammonia was evolved when the sodium hydroxide was added in the preparation of the reagent.

REAGENTS PREPARED FROM ANALYTICAL REAGENT CHEMICALS

Fresh copper reagents were now prepared in which Analytical Reagent chemicals were used throughout. The sodium carbonate was Kahlbaum pro. anal. (Griffin and Tatlock) ; the other chemicals were from British Drug Houses.

Fresh phosphomolybdic acid was prepared using an equivalent amount of sodium molybdate (instead of molybdic acid and sodium hydroxide). 44.3 g. Na_2MoO_4,H_2O and 5 g. $Na_2WO_4, 2H_2O$ were dissolved in 250 ml. distilled water containing 2.8 g. NaOH, and the solution boiled for 30 minutes. Water was added to approximately 350 ml., followed by 125 ml. H_3PO_4 (89 per cent w/v ; sp. gr. 1.75) and the volume made up to 500 ml.

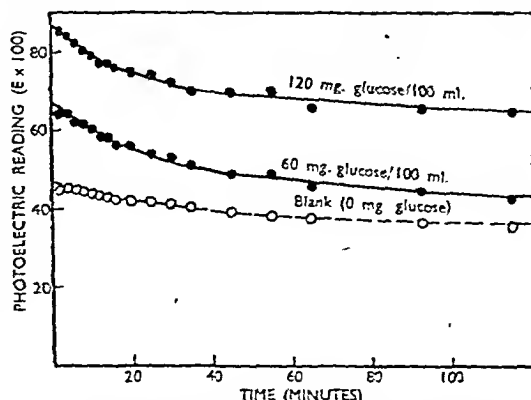*Blank colour.*—The use of these reagents resulted in the elimination of the high blank

FIG. 2.—Photo-electric readings (E × 100) of glucose solutions estimated by the modified Harding copper reagent and the Folin and Wu phosphomolybdic acid reagent. Ordinary quality chemicals—not Analytical Reagent grade. Note the high "blank" readings and the rapid fading of the colours.

readings with the Harding with Folin-Wu combination (Haslewood and Strookman method). Blanks prepared from fresh copper reagents heated with water (instead of glucose solution) gave a deflection of approximately a division on the absorptiometer when the zero adjustment was made with distilled water.

Stability of colour.—Water blanks and standard solutions containing an amount of glucose equivalent to 80 mg. per 100 ml. were prepared, using fresh copper reagents, and the colours developed with either phosphomolybdic or arsenomolybdic acid. The colours obtained were compared in a Hilger Spekker absorptiometer, using an Ilford spectrum red 608 filter. The zero adjustment was made with the blank solutions. Results are shown in Table I. Except in the case of the Somogyi with Folin-Wu combination, the colours remained substantially stable over a three-hour period.

TABLE I
STABILITY OF COLOUR IN AN 80 MG. GLUCOSE STANDARD DURING THREE HOURS
(Figures represent photo-electric readings, $E \times 100$)

Time (mins.)	Harding and Folin-Wu	Harding and Nelson	Somogyi and Folin-Wu	Somogyi and Nelson
2	22.5	24	23	24.5
10	22.5	25	22.5	25.2
20	22.5	25	21.5	26
30	22.5	25	20.5	26
60	22.2	25	19.5	26
180	22	25	17.5	25

The development of colour in a similar series, in which the colour development was followed for eighteen hours and the blank colours were read with a water adjustment of the instrument zero, is illustrated in Table II. In every case, and particularly in the blanks, the colour becomes more intense with the lapse of time ; but the difference between the standard and the blank readings becomes less except in the case of the Harding with Nelson combination, where it becomes greater. Thus, although there is an actual intensification of colour in every case, there is, except with the Harding–Nelson, a " relative fading " (that is, a lessening of the difference between standard and blank readings), which is most marked in the Somogyi with Nelson combination.

Proportionality.—The relation between colour development and concentration of glucose with the fresh copper reagents is shown in Fig. 3. Beer's Law is closely adhered to, even in the low range of sugar values, particularly with the Harding–Nelson combination. The Harding–Folin-Wu and the Somogyi–Nelson combinations also give satisfactory results, but the Harding–Nelson combination is preferred because of the greater stability of its blank colours.

Discussion

The above results emphasize the necessity of preparing sugar reagents from the best chemicals obtainable. Only Analytical Reagent chemicals should be employed. The use of reagents prepared from impure chemicals can only lead to the production of unreliable results.

· The purity of sodium carbonate seems to be the most important factor in the production of satisfactory copper reagents. If Analytical Reagent sodium carbonate cannot be obtained, the ordinary sodium carbonate should be heated in a crucible to red heat and maintained at that temperature for about thirty minutes. The cooled product is powdered in a mortar and stored in a bottle with a tightly fitting stopper. This treatment ensures that any contaminating sodium bicarbonate is decomposed into sodium carbonate. The procedure,

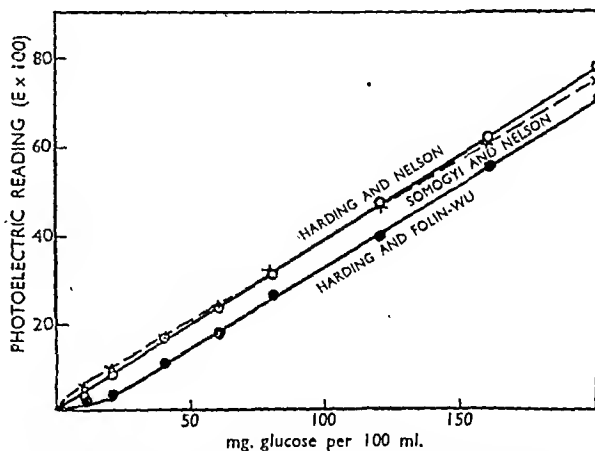FIG. 3.—Calibration curves for glucose methods with reagents prepared from Analytical Reagent grade chemicals. Photo-electric readings ($E \times 100$) with the Hilger Spekker absorptiometer, 1 cm. cells and Ilford spectrum red filters.

however, is only recommended when Analytical Reagent sodium carbonate is not available.

Some samples of Nelson arsenomolybdic acid, prepared strictly according to Nelson's instruc-

TABLE II

STABILITY OF COLOUR OF A 200 MG. GLUCOSE STANDARD DURING EIGHTEEN HOURS

(Figures represent photo-electric readings, E × 100)

Time	Harding and Folin-Wu			Harding and Nelson			Somogyi and Folin-Wu			Somogyi and Nelson		
	200 mg. std.	Blank	Diff.	200 mg. std.	Blank	Diff.	200 mg. std.	Blank	Diff.	200 mg. std.	Blank	Diff.
(min.) 5	63	3.5	59.5	77.5	14	63.5	76	18.5	58.5	80	19.5	60.5
(hr.) 1	64.5	5	59.5	78.5	14.5	64	77.5	22.5	55	83	23	60
18	72	21.5	50.5	127	18	109	78	40	38	107	85	22

tions, give a persistent green colour with either the Harding or Somogyi copper reagents. Other batches give a green colour which with Harding copper reagent develops into a blue colour within 5 minutes, while with the Somogyi reagent the colour retains a definite greenish tint for about one hour. It has also been found that the readings of blanks prepared from the Harding or Somogyi reagents increase slightly with the age of the reagent. Neither of these points affects the accuracy of the results when the readings of the colours are made with a red light filter, and when, with a photo-electric instrument, the zero setting is made with a blank solution.

Summary

1. The Harding copper reagent (without the iodate) proposed by Haslewood and Strookman gave better colour development both with Folin and Wu phosphomolybdic acid and with Nelson arsenomolybdic acid than did the corresponding Somogyi reagent.

2. The colour development obtained with Harding reagent is of the same order as that obtained with Somogyi reagent.

3. The Harding–Folin-Wu and Harding–Nelson combinations are found to give the more stable colours, the Somogyi–Nelson and the Somogyi–Folin-Wu combinations being subject to varying degrees of fading.

4. With low sugar concentrations the Harding–Nelson combination is found to give more reliable results than the Harding–Folin-Wu.

5. Only chemicals of Analytical Reagent (A.R.) quality should be used for the preparation of blood sugar reagents.

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FAMILIAL INTESTINAL POLYPOSIS

BY

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Familial polyposis is of interest for its own sake as a sort of pathological curiosity and also in relation to the problem of cancer. The term "polyposis" is used to describe a widespread adenomatous proliferation of the intestinal mucous membrane, leading to the formation of multiple adenomata. It should not be applied to intestinal polyps of different histological structure, such as inflammatory or fibroid polyps, because these exhibit no special tendency to carcinoma, whereas true polyposis undoubtedly does. In fact, intestinal cancer is an almost invariable sequel to familial polyposis.

Clinical Classification of Adenomatous Polyps

Two clinical types of multiple adenomatous polypi may be distinguished: (1) a *familial* variety which affects several members of one family and generally manifests itself in adolescence; and (2) a so-called *acquired* variety which usually appears later in life and is not hereditary. This classification of intestinal polypi was first suggested by Erdmann and Morris (1925) who pointed out that there are some features in which these two clinical conditions resemble each other and some in which they differ. Both types of polypi tend to malignancy, but in the familial or adolescent variety the polyps are very widely disseminated throughout the rectum and colon, whereas the acquired or adult type is more limited in numbers and extent. Also the liability to cancer is greater in the familial than in the acquired type.

Differentiation of the Familial Type

Although these points of distinction between the two varieties are of value, they do not always serve to settle at once whether a case of multiple polypi is of the familial or acquired type. In most cases this can only be decided by inquiring carefully into the family history. When this is done there is often ample evidence that near relatives have been similarly affected and the case is obviously of the familial type. In other instances there is nothing to suggest that any near relative has had any complaint which could be attributed to polyposis. Our experience at St. Mark's Hospital is that evidence of a familial character is obtained in about one-third to one-half of all polyposis patients at the time of first diagnosis, and this is in close agreement with the experience of McKenney (1936) and Friedell and Wakefield (1943). However, this is certainly an underestimate of the extent of the familial character, because if the distinction between the familial and the acquired type of polyposis is only to depend on the presence of polypi in other members of a family then obviously the first victim in a family will always be classified as "acquired." Only when subsequent cases have occurred among relatives will the familial character be apparent. For this reason cases provisionally classified as of the acquired type may need to be reconsidered if evidence of a familial disposition is discovered later.

Development of Knowledge of Familial Polyposis

Polyposis is a relatively rare disease, and before the closing years of the nineteenth century nothing was known of its familial character or liability to cancer, though a few descriptions of multiple polypi can be found in the medical literature of that period. Some of the reported cases were probably examples of inflammatory polyps following chronic ulcerative colitis or dysentery; in fact, it was generally supposed in those days that all polyps were the late result of an inflammatory process. The credit for recognizing the familial character of some forms of polyposis should be given to Harrison Cripps (1882), who sixty-five years ago read a paper before the Pathological Society of London entitled "Two cases of disseminated polypus of the rectum." The interest of this communication lay in the fact that the two patients, a boy of 19 and a girl of 17, were brother and sister. Within a few years several other examples of familial polyposis were reported, and the fact became established that heredity is an

important consideration in relation to some intestinal polypi.

Eight years later Handford (1890) also presented a paper before the same society in which he described a case of polyposis in a woman aged 34 who died from cancer of the rectum. Similar cases were soon recorded in other countries and evidence began to accumulate both of the familial character and of liability to cancer.

The introduction of the sigmoidoscope resulted in a rapid advance in knowledge of this disease; and Lockhart-Mummery, who was one of the first surgeons in England to advocate the more general use of the sigmoidoscope, was also one of the first to publish genealogical trees depicting the transmission of polyposis from one generation to another (Lockhart-Mummery, 1925). His paper illustrated the inheritance of polyposis in three families, and five years later further information about these families and other similar cases was recorded (Dukes, 1930). Since then many more cases have been reported.

Morbid Anatomy and Histology of Polyposis

In the familial type of polyposis the whole mucosal surface of the colon and rectum is covered with innumerable tumours ranging in size from tiny mammillations, only just visible, up to large pedunculated tumours. The smaller tumours are sessile (Fig. 1), but the larger ones are often attached by a broad strap-like stalk (Fig. 2). The total number of tumours varies considerably. They may cover the whole surface diffusely so that it is hard to find an unaffected patch of mucous membrane, or they may be more sparsely scattered; but there are always large numbers of tumours. The disease generally affects the whole colon and rectum but not the small intestine and stomach, though occasional isolated polyps may be found in the stomach also (Coffey and Bargen, 1939). As a rule, however, the disease ends abruptly in the ileo-caecal junction.

Sections show the tumours to be adenomas in which active epithelial proliferation is in progress. All stages in the development of an adenoma may be noticed, the commonest lesions being small adenomas with a short pedicle (Fig. 3). In between the tumours the intestinal mucous membrane may show patches of hyperplasia.

When examining a case of polyposis a special search must be made for signs of carcinoma. The features which suggest malignancy are darker colour, firmer consistency, and ulceration. The adenomata themselves are often darker in colour than the surrounding mucosa, but a focus of carcinoma makes such a tumour even darker still. Most adenomata feel soft and have a rubber-like consistency, but when malignancy has supervened they feel much harder and more solid. Ulceration is almost invariably a sign of malignancy, and so is induration of the stalk of a pedunculated tumour.

Symptoms and Diagnosis of Polyposis

In familial polyposis the onset of symptoms is usually insidious and there is at first only slight looseness of the bowels associated with the passage of small quantities of blood and mucus. At first the patient is not ill in the clinical sense. This is a point of distinction between polyposis and polypoid lesions of an inflammatory nature following colitis or dysentery, because in these the initial illness is of a severe character. On the other hand, with familial polyposis the initial symptoms are relatively trivial. For instance, in two cases recently encountered the patients had suffered from symptoms of polyposis for seven years before consulting a doctor.

The final diagnosis of polyposis generally depends on the result of sigmoidoscopic examination, but radiographs are sometimes a valuable aid to diagnosis. Anderson and Marxer (1930) have pointed out that in polyposis the mucosa in general presents a mottled or honeycombed appearance, but frequent examination may be needed to demonstrate this well, and the general routine of the ordinary barium meal may require some modification.

Inheritance of Polyposis

It is well known that, although cancer itself is not inherited in the ordinary sense of the word, none the less certain families do show a strong predisposition to the development of tumours. In these families tumours of a specific type tend to appear in a specific organ (Macklin, 1935). The simple occurrence of two or more cases of cancer in one family does not of course prove that any inherited predisposition exists. The operation of the law of chance will naturally result in some families having an excess of cancer and others none at all. But, as Weller (1937) has pointed out, there is ample evidence of the existence of some families in which the mass incidence of cancer is significantly in excess of all normal expectations, and he quotes as an example the family originally studied and reported by Warthin. Forty-one out of 174 members of this family attaining the age of

25 years developed neoplasms. With two exceptions all the cancers were of the gastro-intestinal tract and uterus. No less than 20 of the males had suffered from cancer of the intestinal tract.

Members of polyposis families show no special proclivity to the development of tumours in any other organ of the body than the intestine, and the disease does not usually manifest itself before childhood or puberty although the age at which adenomata develop varies considerably in different families. For the cases we have investigated at St. Mark's Hospital, London, the average age at which adenomata were discovered was 22 years. The youngest patient was 8 and the oldest 45. There was reason to believe that the tumours had been present for a year or two before they were discovered, so they probably developed at about 20 years of age on the average. Gabriel (1945) states that only rarely has polyposis been noticed in the first decade or after forty years of age. Occasionally, however, cases have been reported which suggest that polypi have been present at birth or developed in infancy. Thus McKenney (1936 and 1939) reports one family in which he sigmoidoscoped four children aged 2, 5, 9, and 11, and found adenomas in each. These tumours seemed to be progressively larger and more numerous as age advanced, and he considered that in these four children polypi had probably been present at birth.

One of the unsolved problems in the pathology of polyposis is whether all or only some members of a family inherit the mysterious defect which manifests itself finally as polyposis. In a decent-sized, intelligent, and co-operative polyposis family one can generally get evidence that about half the adult members are or have been affected. The question is whether the at present unaffected individuals are destined to develop the disease later. The only way to answer this conundrum is to watch to see what happens to them. At St. Mark's Hospital we have engaged in this waiting game for more than 20 years and have had the opportunity more than once to observe polypi develop in an individual who at the first sigmoidoscopic examination was certified free from polyps.

A striking example of this is seen in the genealogical tree recorded in Fig. 4. In this chart males are represented by squares, females by circles, and individuals with intestinal tumours (whether adenomas or carcinomas) by black squares or circles respectively. The man recorded as III 1 (indicated by an arrow) is now 60 years of age. His father, grandfather, and at least five of his uncles and aunts died of cancer, and some of them had transmitted polyposis to their descendants. This patient was first examined by Mr. Lockhart-Mummery at the age of 38 and was found to be free from polypi. He was kept under observation, and six years later, when he was 44, polypi were first noticed. The patient was quite well aware of the existence of the skeleton in the family cupboard and he kept closely in touch with his medical advisers, which proved to be a good policy for him, because at the age of 56 he developed cancer of the descending colon. This was successfully excised by Mr. Lockhart-Mummery, but three years later at the age of 59 he developed cancer of the rectum, which was dealt with by Mr. Naunton Morgan by perineo-abdominal excision. A few months later, at the age of 60, the patient developed a third cancer at the site of his colostomy, and this was also removed without delay.

This patient's story illustrates the difficulty of deciding whether the defect leading to polyposis is inherited by all or only by some members of a family. On the first occasion when the pedigree of this patient was published (Lockhart-Mummery, 1925) he was represented as unaffected. When further details were published by Lockhart-Mummery and Dukes in 1939 he was recorded as suffering from polyposis. Now the story has unfolded itself still further, and he is recorded as an example of multiple cancer.

This incident also illustrates the fact that a polyposis family group is constantly changing its composition and the proportion of unaffected individuals varies from time to time. McKenney (1939), who has kept careful records of three families for several years, has described tersely the vigilant attitude which must be adopted by the student of the disease. " A polyposis family group history is an ever-changing one," he says, " and the chronicler must not lay aside his typewriter as the story must constantly be rewritten."

There are two ways in which further knowledge may be gained about the transmission of polyposis. One is by patiently observing year by year and recording what happens in polyposis families. The other is by searching for curious and unexpected incidents which may throw light on the problem.

There is, for instance, the family reported by Zahlman in which a woman free from polyposis was married twice (for reference see Dukes, 1930). By her first husband, who was also free from polyposis, she had two children, both of whom were healthy. Her second husband suffered from polyposis, and ultimately died of cancer. By him she had four children, all of whom suffered from polyposis. This proves transmission by the male.

Again, in a family recorded by McKenney (1936), a woman suffering from polyposis was married twice, each of her husbands being free from the disease.

She had four children, two by each husband, and all four children suffered from polyposis. This proves transmission by the female.

One of the families studied at St. Mark's Hospital is of special interest because of the presence of twins in the second generation. Each of the twins developed polypi about the age of 30. One was treated by colectomy at the age of 41 and succumbed to the operation : the other also died of intestinal cancer at the age of 41.

Polyposis patients sometimes ask the question : Who was responsible for first introducing this defect into our family and what is it due to? This, of course, is another unsolved problem, but a clue to it might be obtained if family pedigrees could be traced further back. People generally know something about the health of their parents, but very little about their grandparents and practically nothing about their great-grandparents, so it is seldom possible to trace a complaint through a family for more than two or three generations.

When further data are available it may possibly be found that polyposis results from the chance mating of two individuals, each of whom was destined to die of cancer of the intestine. At any rate it is worth recording that in two of the ten families we have investigated at St. Mark's Hospital the disease appeared in a family in which both father and mother had died of cancer of the rectum or colon. As far as could be ascertained this was the starting point of the disease in these two families.

Relationship of Polyposis to Carcinoma

Malignant disease secondary to familial polyposis is characterized by its early age of onset and the fact that more than one primary focus of carcinoma may be present. Among the general population it is rare for cancer of the rectum or colon to develop before the age of 40, but in families affected by polyposis it develops in the thirties or even earlier. The age at death from intestinal cancer in polyposis families is younger by about twenty years than the average age of death from intestinal cancer in the general population. There is considerable variation in different families, but in most cases it may be said that polypi may be expected before the age of 20 and cancer is liable to manifest itself some ten to fifteen years later.

Polyposis is not a familial disease which is likely to spread and become more prevalent : on the contrary polyposis families tend to die out. That is because cancer so frequently develops before the age of marriage and because even those who do marry die young. Moreover there seems to be a tendency for malignant disease to begin at an earlier age-period in each succeeding generation.

The treatment of polyposis presents a difficult problem. Constant medical supervision is necessary so that a radical excision may be undertaken as soon as malignancy is detected. No other method of treatment than surgery has any permanent effect on the course of the disease.

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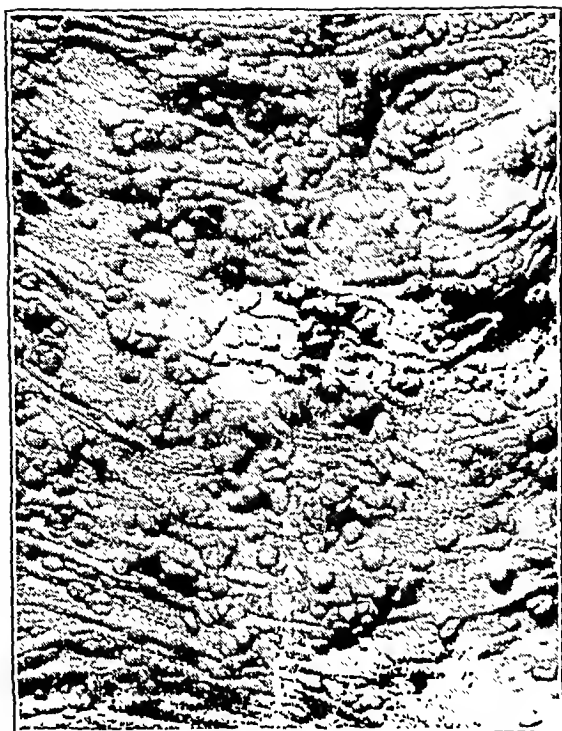
FIG. 1.—Surface view of mucous membrane of colon in familial polyposis, showing sessile adenomata.
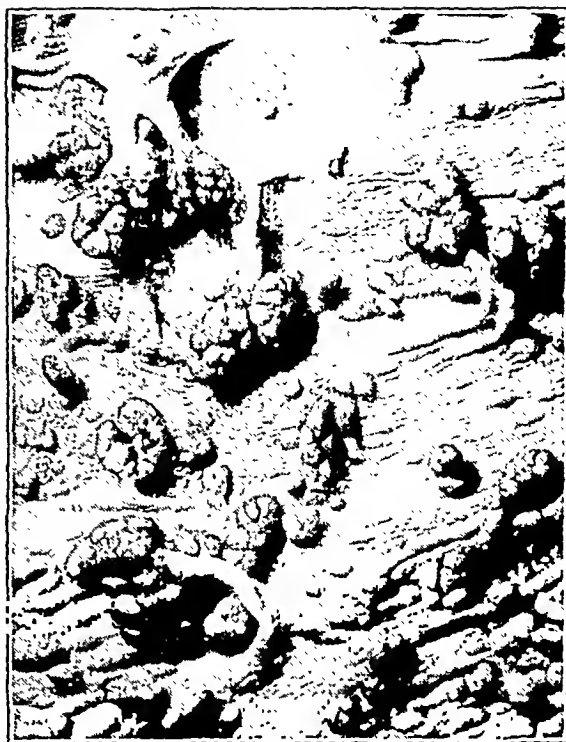
FIG. 2.—Pedunculated adenomata in familial intestinal polyposis.

FIG. 3.—Section through rectal mucosa in a case of adenomatosis (×8). Reproduced by kind permission of the *Lancet*, from the article by Lockhart-Mummery and Dukes (1939).
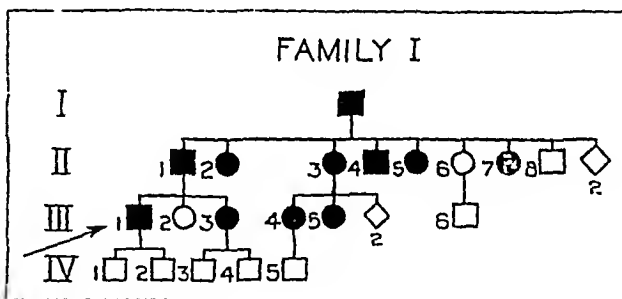
FIG. 4.—Family tree, showing: I Death from cancer of rectum, aged 42 ; II (1, 2, 3, 4, 5, 7) Death from cancer of rectum, aged 42, 52, 54, 39, 27, and 44 years respectively ; III (1) Polypi at age 44, carcinoma of splenic flexure at 56, carcinoma of rectum at 59, and carcinoma in colostomy at 60 years ; (3) Polypi at age 37 ; (4) Polypi at age 11: colectomy for polyposis at 31: death from cancer of rectum at 52 ; (5) Death from cancer of colon aged 33 years.

REPORT ON TWO SPECIES OF THE GENUS FUSIFORMIS OBTAINED FROM PATHOLOGICAL LESIONS IN MAN

BY

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It is at present very difficult to classify accurately a given strain of the genus *Fusiformis*, and the following case histories may therefore be of interest.

Clinical Histories

Strain 1.—A woman aged 46 years was admitted to Shrodells Hospital, Watford, Hertfordshire, under the care of Dr. Duffus on Jan. 12, 1945, complaining of severe abdominal pain. Since Dec. 25, 1944, she had noticed frequency and pain on micturition. Her periods had been normal and bowels regular. Abdominal pain had become severe on Jan. 8. Her own doctor had administered 15 g. sulphonamide; chemotherapy was discontinued in hospital.

On admission, her temperature was 98° F.; pulse 112. There was extreme tenderness in the left inguinal region. Vaginal examination revealed fullness in the left fornix. The urine was normal. The blood showed leucocytosis and a "shift to the left" (red blood cells 5,090,000 per 100 c.mm. of blood; Hb 70 per cent; colour index 0.7; white blood cells 17,200; polymorphs 75 per cent, band forms 15 per cent; eosinophils 2 per cent; basophils 1 per cent; lymphocytes 18 per cent; large mononuclears 4 per cent.

A laparotomy was performed by Dr. Duffus on Jan. 12, 1945. One pocket of excessively foetid pus was found in the pouch of Douglas, and another pocket on the outer surface of the descending colon at the level of the iliac crest. The left Fallopian tube was dilated and inflamed. There was no diverticulitis or other abnormality of the abdominal viscera. A drainage tube was left in situ and the abdomen closed. There was free drainage of pus for several days. The patient made an uneventful recovery and was ready for discharge on Feb. 2, 1945.

Bacteriological examination of pus from the pelvic abscess.—Direct examination showed degenerate pus cells, numerous Gram-negative filamentous bacilli, and a few Gram-positive cocci in chains. The material was inoculated on to two horse-blood agar plates containing 6 per cent agar (Hayward and Miles,

1943), and on Nagler plates (Hayward, 1943), and into a tube of Robertson's cooked-meat medium. The Nagler plate failed to show the presence of *Cl. Welchii* or *Cl. bifermentans*. One blood-agar plate incubated aerobically remained sterile; the second blood-agar plate, incubated anaerobically, when examined after seventy-two hours showed (1) numerous colonies, 0.2 to 0.5 mm., opaque and slightly haemolytic, which were found to be composed of Gram-negative pleomorphic cells; (2) seven-minute "dewdrop" colonies (in the pool), which finally conformed to Prevot's classification of *Str. micros*. The cooked-meat medium yielded *Ps. pyocyanea* in addition to these organisms.

Because of the resemblance of the anaerobic Gram-negative bacilli to those seen in profusion in direct smear, they were considered to be the cause of the abscess.

Strain 2.—A woman aged 62 years was admitted to Shrodells Hospital under the care of Dr. Roberts on July 4, 1945; she complained of a painless swelling in the region of the pubic symphysis which she had had for 3 or 4 days before admission. During this time she had also had diarrhoea. Her temperature was 102.6° F. The lump in her pubic region was situated superficially, was 3 inches in its largest diameter, and was surrounded by a zone of erythema. She was placed on full dosage of sulphathiazole and her temperature rapidly fell to normal. On July 6 the abscess was opened and a large gangrenous slough removed. At the same time pus was taken for culture. The abscess healed well, and on Aug. 7 was only discharging slightly. On July 30 a radiograph of the pelvis failed to reveal any bony abnormality.

Bacteriological examination of the pus.—Direct examination showed pus cells, numerous Gram-negative bacilli, and a few Gram-positive cocci. The material was inoculated on to two horse-blood agar plates, containing 2 per cent agar, one of which was incubated aerobically and the other anaerobically. On the aerobic plate there was a scanty growth of three types of colony. One of these was a beta-haemolytic streptococcus which did not belong to Lancefield's

groups A, B, C, or G. The other two were diph-theroids.

On the anaerobic plate, after forty-eight hours' incubation at 37° C., there was a heavy growth of colonies about 0.6 mm. in diameter, consisting of small Gram-negative bacilli. In view of its preponderance and the fact that the streptococcus did not belong to a Lancefield's group that is normally pathogenic in man, this anaerobic organism was probably the principal pathogen. As regards the remaining organisms isolated from the pus, the Gram-negative anaerobic bacillus was later found to demonstrate satellitism to one of the diphtheroids obtained from the aerobic plate.

Description of the Two Strains of Gram-negative Anaerobic Bacilli

Morphology.—The bacilli were grown on a Fildes slope and incubated in an atmosphere of 5 per cent CO_2 and hydrogen for seventy-two hours.

Strain 1.—The cells varied considerably in length, being $0.5 \times 1.0\text{-}10.0 \ \mu$. They were Gram-negative bacilli and showed irregular staining, and globoid or oval bodies, $1.3 \ \mu$ in diameter, lying free or along the bacilli, swollen-ended bacilli, or filaments having a central distension. Many long filaments were curled upon themselves. Some were branched, but it was difficult to tell whether this was true or false branching. Cocco-bacillary forms

Strain 1, showing filamentous forms and globoid bodies when grown on enriched media. ($\times$ 1,000.)

occurred in unenriched media. The organisms were non-motile, this being confirmed by flagella staining; non-capsulated; and did not contain spores (a culture did not survive heating for ten minutes at 80° C.). The organism was not acid-fast.

Strain 2.—The organism was a small Gram-negative bacillus, with parallel sides and rounded ends, about $0.2 \times 0.6\text{-}2.5 \ \mu$. It stained uniformly with carbol fuchsin, showing little pleomorphism

and no branching. No capsules or spores were demonstrated, and it was not acid-fast. In Fildes broth incubated under the same conditions there was rather more pleomorphism with coccal and long filamentous forms up to 10 μ in length. Large globoid bodies or distensions in the filaments were never seen. The organisms were non-motile, as confirmed by flagella staining.

Colonial morphology.—Colonies were grown on blood-agar plates containing 2 per cent agar and incubated at 37° C. for seventy-two hours in a microaerophilic atmosphere (i.e., the pressure in a jar was lowered to 260 mm. Hg and the vacuum replaced with hydrogen).

Strain 1.—The colony was between 1 and 1.5 mm. in diameter, grey, circular, with an entire edge, semi-translucent, slightly haemolytic, but never frankly beta-haemolytic, raised, convex, smooth, shiny, compact, of a butyrous consistency, and easily emulsifiable.

Strain 2.—This was similar to strain 1, but no haemolysis was present.

Growth requirements

Strain 1.—After recent isolation, the strain failed to grow in peptone water, broth, or gelatine agar. The addition of Fildes extract in a concentration of 1 per cent yielded prolific growth. Peptone water with V factor, with X factor, and with V and X factors yielded growth. Satellitism to a *Staph. albus* colony on an agar plate was marked after seventy-two hours' incubation. About six months after isolation the organism was found to be capable of growing in peptone water and broth.

Cultures incubated in 5 per cent carbon dioxide in hydrogen, under microaerophilic conditions and under anaerobic conditions yielded colonies whose mean diameters were 0.60 mm., 0.44 mm., and 0.08 mm. respectively. No growth was obtained in 5 per cent carbon dioxide in air, or aerobically. Fildes-agar shake cultures showed a heavy band of growth 12 mm. below the surface of the agar. Smaller colonies grew right to the bottom of the tube, but no colonies developed above the band. For up to five days the colonies were translucent, lenticular, and gave the appearance of gas bubbles; later they became opaque and the agar was split by gas.

Strain 2.—This was similar to strain 1, except that poor growth was obtained in peptone water soon after isolation. Addition of Fildes extract or V and X factors increased the amount of growth. In the Fildes-agar shake culture, growth was maxi-

TABLE I

	Strain 1	Strain 2	F. necrophorus	F. fragilis
Indole production	−ve	−ve	+ve	variable, usually −ve
Lactose fermentation ..	+ve	+ve	−ve	+ve
Glucose ,, ..	+ve	+ve	+ve	+ve
Sucrose ,, ..	+ve	₁+ve	variable, usually −ve	÷ve
Maltose ,, ..	+ve	+ve	+ve	+ve
Mannitol ,, ..	−ve	−ve	variable, usually −ve	−ve
Fructose ,, ..	+ve[1]	+ve[1]	+ve	÷ve
Glycerol ,, ..	−ve	−ve	variable	
Gas production in carbohydrate media	little	little	much	little
H₂S production[2]	+ve	+ve	+ve	−ve
Nitrate reduction	−ve	−ve	−ve	−ve
Litmus milk	Acid and clot after 7 days		No change	Some strains; acid and clot
Gelatin liquefaction	−ve	−ve	−ve	−ve
Liquefaction of inspissated serum	−ve	−ve	−ve	−ve
Catalase production	+ve	+ve		

[1] Acid but no gas in fructose.
[2] Growth on a Fildes slope tested by blackening of a lead acetate paper; ÷ve 35 mm. in 24 hours.

TABLE II

Date	Animal	Mode of inoculation	Killed	Enlarged glands		Organism recovered on culture from				Titre
				Inguinal	Sublumbar	Local lesion	Gland	Heart blood	Spleen	
*7.7.45 20.3.45	Mouse	Intramuscular aa 5% CaCl₂ and cooked-meat culture	9.7.45 26.3.45	+ +	÷ ÷	÷ ÷	− ÷	− −	− −	
20.3.45	Mouse	Intramuscular 0.5 ml. cooked-meat culture	26.3.45	+	÷	−	−	−	−	
*31.7.45 20.3.45	Mouse	Intraperitoneal 0.2 ml. cooked-meat culture	10.8.45 26.3.45	− −	÷ +	− −	− −	− −	− −	
26.3.45	Mouse	Intramuscular aa 5% CaCl₂ and cooked-m at culture	14.4.45	+	+	÷	÷	−		
20.3.45	Guinea-pig	Intramuscular 1.0 ml. cooked-meat culture	14.4.45	−	−	−	−	−	−	20
26.3.45	Rabbit	Intralabially. Left lip, 0.2 ml. CaCl₂ + 0.2 ml. saline Right lip, 0.2 ml. CaCl₂ + 0.2 ml. culture	30.5.45	−	−	−	−	−		< 2
26.3.45	Rabbit	Intravenous 1.0 ml. cooked-meat culture	30.5.45	−	−	−	−	−	−	16
	10 normal human sera									<20

* = Strain 2.

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mal at 7 mm. below the surface, but no gas was produced. Colony size was increased in an atmosphere containing carbon dioxide as much as with strain 1.

In Fildes broth both strains produced an even turbidity with a slight ropy deposit after forty-eight hours.

Strain 1 survived three weeks in cooked-meat medium.

Heat resistance.—Both strains failed to survive five minutes at 60° C.

Biochemical activities.—These were tested with strips of iron in sugar tubes and the results are listed in Table I.

Drug sensitivity.—Both strains were sensitive to 5 mg. per cent sulphadiazine, sulphamezathine, sulphapyridine, sulphathiazole, and sulphanilamide (Harper and Cawston, 1945). The minimal bactericidal concentration of penicillin for strain 1 was 62 units per ml., while for strain 2 it was between 10 and 20 units per ml.

Biological tests.—From the biological tests of strain 1, listed in Table II, it will be seen that the strain proved completely non-pathogenic to the laboratory animals used. Mice receiving intramuscular inoculations of 0.25 ml. of 5 per cent calcium chloride with 0.25 ml. cooked-meat culture showed enlargement of their proximal glands. From these glands the fusiform organism was recovered on culture. Because of the morphological resemblance of this bacillus to *F. necrophorus*, a rabbit received intralabial inoculation. No necrosis developed during a two months' period of observation.

Serological examination of rabbits and a guinea-pig failed to reveal any agglutinin response to the organism inoculated ; similarly ten sera, sent to the laboratory for Wassermann test, failed to agglutinate a suspension of the test strain in a final dilution of 1 in 20.

In the case of strain 2, only mice were tested. They received intramuscular injection of the culture with calcium chloride and intraperitoneal injection with cooked-meat culture.

Discussion

Organisms of the genus *Fusiformis* are the predominant flora of the gastro-intestinal tract, and from faeces a very large variety of strains can be obtained with widely variable characteristics (Eggerth and Gagnon, 1933). Other members of the group cause suppurative lesions of mucous membrane and abscesses in tissues close to the gut, the vagina, and the genito-urinary system. They have been studied for many years from the bacteriological and clinical standpoint by French workers (Lemierre, 1936, who gives the main French references) and more recently in America (Dack, 1940, who gives a fairly full bibliography). In all probability infections with these organisms are more common than is realized in this country.

Of the various described species the following two have been studied most completely and are most closely related to the strains we have isolated. However, lack of uniformity in technique, inadequate description of individual strains, and conclusions based on the study of only a few strains make comparisons of different workers' results difficult. The first species, name *Fusiformis necrophorus* (Topley and Wilson, 1936), Schmorl's bacillus (Weinberg and others, 1937), *Bacteroides necrophorus*, etc., is probably identical with *Bacillus funduliformis* (Weinberg and others ; Dack and others, 1938). Fairly full descriptions have been given by Henthorne and others, 1936 ; Tessier and others, 1931 ; and by Dack and others, 1938. All authors agree that, morphologically, this is a non-motile, Gram-negative bacillus, usually ovoid, with biopular staining in exudates, and, in culture, highly pleomorphic, with long filamentous forms, often swollen. There are often large globoid bodies, irregular staining of the filamentous forms, and frequently false branching. The significance of the large spheroidal bodies found in cultures of "*Bacteroides funduliformis*" is discussed by Dienes and Smith (1944). They interpret them as a sign of a reproductive process different from binary fission. However, throughout a series of papers on the subject they do not appear to have investigated their strains apart from the morphology and growth requirements. In the full descriptions by the other authors quoted there is agreement that colonies are constantly haemolytic. Tessier and others describe haemolysis in blood glucose agar shake cultures resembling that of a beta-haemolytic streptococcus, while Dack and others state that on first removing a blood-agar plate from an anaerobic jar there is no haemolysis. As soon, however, as the blood becomes oxidized there is a wide area of green discoloration round the colonies, which usually changes to clear haemolysis on standing.

It appears that in liquid media there is a growth at the bottom of the tube and a clear supernatant fluid.

As regards biochemical activities, Table I illustrates the results obtained by these authors. No strains investigated fermented lactose. Some strains appear to be able to produce gas and a little acid in carbohydrate-free basic media, which may account for some of the discrepancies. All strains produce gas as well as acid with fermentable carbohydrates.

The organism is often pathogenic to rabbits, causing, on subcutaneous injection, an abscess which may spread and finally kill the animal with metastatic abscesses in the lungs. Intravenous injections may cause abscesses in the lungs, liver, and joints. Reports differ as to the pathogenicity of all strains. Those obtained from liver abscesses in cattle are more pathogenic than those from lesions in man. The mouse is less susceptible than the rabbit, while the guinea-pig is immune.

The second species is called *Fusiformis fragilis* (Topley and Wilson, 1936). It has been described by Henthorne and others (1936), and by Cohen (1932). In contradistinction to the first group, the organism is not pleomorphic and resembles a coliform bacillus in its morphology. The large spheroidal forms characteristic of necrophorus are not found. The colonies are usually smaller than those of necrophorus and are not haemolytic. In broth there is uniform turbidity. The biochemical reactions are given in Table I. In rabbits subcutaneous injection causes a local abscess, but in general the organism is less pathogenic than necrophorus.

The main distinction between necrophorus and fragilis is usually based on morphology, haemolysis, and the production of indole and hydrogen sulphide, but it appears to us that the fermentation of lactose is also important. However, in view of the fact that the pleomorphism of necrophorus is often present on certain media only, we feel that morphology is an uncertain criterion for differentiation.

As regards the two organisms described by us. No. 1 is similar to fragilis in all points except that of hydrogen sulphide production and of its morphology, which is typical of necrophorus. Methods of testing hydrogen sulphide production used in the reported descriptions of fragilis and necrophorus vary considerably in sensitivity, so that we

doubt the significance of this difference. Furthermore, this organism would have had a morphology typical of fragilis had it been investigated in unenriched media alone, so that we are led to doubt the significance of a distinction between necrophorus and fragilis based on morphology alone. The haemolysis described for necrophorus is far more intense and of a character different from that observed in strain 1.

Strain 2 fits with the description of fragilis given above, again with the exception of hydrogen sulphide production.

Of the remaining members of the genus *Fusiformis, F. fusiformis* (Topley and Wilson, 1936) is separated off on the basis of having pointed ends, and *F. serpens* on motility and liquefaction of gelatin. There are other strains incompletely described.

In conclusion we must emphasize that the full importance of infections caused by these organisms will not be recognized until methods of anaerobic culture are used on a much wider scale than before. Few people realize that anaerobic technique is little more difficult than careful work with aerobic bacteria.

Summary

Two bacterial strains isolated from pathological lesions in man and belonging to the fusiform genus have been described, and their position in regard to the classification of this group has been discussed. They are most closely allied to the species *F. fragilis*.

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DIETHYLENE GLYCOL POISONING:
REPORT ON TWO CASES

BY

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Few cases, if any, of death from drinking diethylene glycol have been reported in this country. This substance is, of course, largely used as an anti-freeze fluid in radiators of motor-cars, and is sold under a variety of trade names in· this country, in America, and probably elsewhere, without any restrictions. There is little or no mention of the toxicity of this substance in British literature. It is not referred to in the latest editions of either Sidney Smith's (1943) or Glaister's (1945) textbooks, nor in Lucas's (1945) *Forensic Chemistry*. There is no mention of this substance in Hunter's (1943) monograph on "Industrial Toxicology." Bamford (1940) refers to the American "elixir" disaster referred to below. Brekke (1930) reported two fatal cases following the drinking of anti-freeze mixture.

An editorial article in the *Journal of the American Medical Association* (1937) describes and investigates a number of deaths in persons taking an "elixir of sulphanilamide." In all, seventy-three persons died as a result of taking this substance. The elixir was a mixture of 9–10 g. of sulphanilamide dissolved in 100 ml. of diethylene glycol. The dose recommended was three teaspoonfuls every four hours for one or two days. The most obvious symptom was anuria. Necropsy revealed a purplish mottling of the kidneys with necrosis in severe cases. It has been suggested by the American investigators of this disaster that sulphanilamide and diethylene glycol may produce additive toxic actions when given in combination.

Boemke (1943) reported an incident in which anti-freeze mixture was substituted for water in making coffee in a German military unit. Many men were dangerously ill and one died. It was suggested that death was due to uraemia caused by the oxidation of ethylene glycol to oxalic acid, which is deposited in the kidneys.

Pons and Custer (1946) describe details concerning ten soldiers who drank anti-freeze solution of ethylene glycol (Prestone type). Apparently this substance was drunk as a substitute for alcohol. They suggest that the minimum lethal dose is about 100 ml. The subjects were all young males. Death occurred in from twenty-two to forty-four hours after ingestion of the fluid. There was little opportunity to study clinical manifestations as all these cases were discovered in deep coma. Post-mortem findings showed generalized congestion of all organs and marked pulmonary oedema. The kidneys were swollen and displayed prominently engorged vessels. Crystals of calcium oxalate were prominent in microscopical preparations of the kidneys, mainly in the tubules. The brains were engorged and showed what the authors describe as "well developed encephalitis." They found numerous oxalate crystals in and about the engorged vessels of the brain. They suggest that coma and subsequent death were due largely to the cerebral complication. For this reason they also suggest that Brekke's two cases (1930) survived, not—as the author suggests—because there was unilateral decapsulation of the kidney, but rather because the amount of ethylene glycol they took was sublethal.

Milles (1946) reported a case of a man aged 30 who drank from a car radiator about 500 ml. of fluid that had had anti-freeze mixture added. The man died ten hours later with symptoms of heart failure and oedema of the lungs. At necropsy the kidney tubules were found to be swollen and disorganized. There were abundant deposits of calcium oxalate crystals in the tubules. He suggests, also, that the cause of death in ethylene glycol poisoning is essentially poisoning from oxalic acid, and cases should be treated as if that substance were the causal agent.

Accounts of the toxicology of the glycols are conflicting and not very precise. Page (1927) says he drank 15 ml. of ethylene glycol without ill effect.

Keston and others (1937) studied the toxic doses of diethylene glycol on rats and rabbits. A dose of from 1 to 2 ml. per kg. of diethylene glycol in rats was required to produce pathological changes, which consisted of extensive injury to the epithelium of the renal tubules, leading to urinary obstruction and uraemia. Holck (1937), in experiments on rats, found that 20 per cent of commercial diethylene glycol in their water killed all rats in about two weeks, and even 10 and 15 per cent proved fatal to some rats. Laug and others (1939) stated that ethylene glycol caused congestion and haemorrhage of the lungs. Smyth and others (1941) used rats and guinea-pigs. The animals were given the very large dose of 50 g. per kilo of various glycols by stomach tube. Most animals died within two days. The lethal dose of diethylene glycol appeared to be about 20 g. per kilo for rats.

Morris and others (1942) fed rats with ethylene and diethylene glycol at levels of between 2 and 3 per cent. The outstanding lesions were large stones in the bladder, tubular atrophy, and renal oxalate concretions. Most of the animals survived a two-year period of feeding. Sollmann (1942) described ethylene glycol as being twice as toxic as propylene glycol and half as toxic as diethylene glycol. Werner and others (1943) subjected rats to repeated exposures of vapours of ethylene glycol and related substances. The concentrations used were not enough to produce any obvious degeneration of the kidneys. Lehmann and Flury (1943) state that the effects of glycol poisoning are due to the formation of oxalic acid in the kidneys.

Case Reports

Case 1.—The fatal case now described was a young man aged 25, a German prisoner-of-war. He appeared back in camp with a bottle of fluid, saying that it had been given him as a present by a civilian. At the inquest later no light was thrown on how the man obtained this fluid. One evening he drank a good deal, perhaps the best part of half a winebottle, and offered some to others ; in fact two other prisoners had a little.

He was admitted to the City Hospital, Plymouth, and died about twenty-four hours after drinking the fluid. It was found later that the fluid in the bottle was pure diethylene glycol. On admission he was in a deep coma and deeply cyanosed.

Necropsy.—The body was that of a well-nourished very muscular young man. The face was deeply cyanosed and blood-stained, with froth oozing from the mouth and nose. The heart muscle was softer than to be expected in a healthy young man. The lungs were intensely congested and oedematous. The mucous membrane of trachea and bronchi were bright red in colour and full of blood-stained mucus. The liver was pale and fatty in appearance. The stomach and intestines showed no lesions. The kidneys were deep red and of a greasy appearance, and showed great engorgement of cortical vessels. The brain was very congested but showed no other abnormalities to the naked eye. The bladder was distended. The urine contained a cloud of albumin, and a few leucocytes and red cells were present.

Microscopically all the liver cells showed cloudy swelling, and there was a slight amount of fatty degeneration. The kidneys showed intense tubular degeneration and swelling ; hardly any nuclei were visible. In the lumen of numerous tubules crystals were prominent.

At first sight the appearances were those that would be expected in a case of poisoning by methyl alcohol, but by the time the necropsy was performed it was known that he had in fact drunk diethylene glycol, and particular attention was paid to the residual urine in the bladder and to the condition of the kidneys. At this time there had been no opportunity of surveying the literature, and it is regretted that a detailed microscopical examination was not made of the brain in view of Pons and Custer's observations referred to above.

Case 2.—The second man who also drank some of the fluid, perhaps 2 or 3 mouthfuls, was a healthy young man of 28 ; he had been slightly sick during the night following. He also was admitted to the City Hospital, Plymouth. On admission his colour was good but the tongue was furred ; he had slight headache and some vomiting shortly after admission. He passed 27 oz. of urine during the night. The next day he felt better and was allowed up. On the sixth day after admission, however, he had repeated fits, his blood urea was found to be 390 mg. per 100 ml., and there was almost complete anuria ; now that the cause of death, the nature of the fluid drunk, and the post-mortem findings of the first case were known, it was decided to perform decapsulation of the kidneys. This was done by the Medical Superintendent of the City Hospital, Mr. G. Larks.

Operation.—At operation the capsule was stripped from each kidney. There was considerable peri-renal oedema. Each organ was enlarged, congested, and bluer than normal. The tension inside the capsule was such that on incision of the capsule the kidney bulged quite markedly, the capsule stripped without

assistance, and the surface of the kidney became immediately redder.

The anaesthetic employed was a "high spinal," using novocain with pentothal, nitrous oxide, and oxygen.

Progress.—The man steadily improved and made an uneventful and perfect recovery. The blood urea findings were:

Just before operation, 390 mg. per 100 ml.
2 days after operation, 310 mg. per 100 ml.
3 days after operation, 210 mg. per 100 ml.
5 days after operation, 105 mg. per 100 ml.
10 days after operation, 30 mg. per 100 ml.

Case 3.—The third case was also a healthy young man, who is said to have had just a taste of the fluid. On admission to the City Hospital his condition was described as good, and he was in fact admitted only as a precaution. He had a little vomiting and headache during the night following the drinking of the fluid. There was a considerable amount of albumin in the urine, but no cells or casts. His blood urea on admission was 90 mg. per 100 ml. He made an uninterrupted recovery.

Comment

Without doubt diethylene glycol is a very toxic substance : if any general tendency develops to drink it as a substitute for alcohol, the results may be disastrous. The range of the lethal dose in man seems to be quite unknown.

Methods of treatment require investigation, and it has still to be decided whether or not treatment should be that for oxalic acid poisoning ; it seems certain that the kidneys bear the brunt of the damage.

It would seem from the second case quoted in this paper that in very severe cases decapsulation of the kidneys may be a life-saving measure.

In view of Pons and Custer's findings, further observations on the histology of the brain should be undertaken.

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ABSTRACTS .

(In this section of the Journal some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.)

BACTERIOLOGY

The Effect of Water Soluble Lipids on the Growth and Biological Properties of Tubercle Bacilli. DUBOS, R. J., DAVIS, B. D., MIDDLEBROOK, G., and PIERCE, C. (1946). *Amer. Rev. Tuberc.*, 54, 204.

The slow rate of growth of tubercle bacilli in the conventional fluid culture media has been overcome by the use of a synthetic basal medium to which is added 0·05% " tween 80 " and 0·1 to 20% filtered sterile serum albumin (conveniently 0·25% bovine serum fraction V, prepared as a powder). " Tween 80 " is a polyoxethylene derivative of sorbitan mono-oleate, which is water-soluble and heat-stable. Inoculation of 5 ml. of this medium in 25-mm. test-tubes leads to rapid diffuse submerged growth in response to very small amounts of tubercle bacilli. It is believed that the " tween 80 " acts as a wetting agent and that oleic acid is split off by a bacterial lipase to provide a readily available foodstuff; free oleic acid is, however, toxic, but this toxicity can be neutralized by the albumin. By adding 1·5% agar to this fluid medium and using 0·5% albumin, a solid medium is obtained that will give surface colonies from inocula in 1 to 2 weeks. Tubercle bacilli growing diffusely in the liquid medium retain their typical morphology and staining characteristics; they are viable for long periods and remain fully virulent. Such bacilli can be used for inoculation into experimental animals and into chick embryos, and they can produce, as well as react specifically with, immune sera. The medium can also be used for primary isolation of tubercle bacilli from pathological material.

[The use of water-soluble lipids is likely to revolutionize the techniques of culture of *Myco. tuberculosis.*]

P. D'Arcy Hart.

Stevens-Johnson Syndrome. Report of a Case. NELLEN, M. (1947). *Lancet*, 1, 326.

A 20-year-old male was admitted to hospital with a 4 days' history of severe malaise, sore throat, productive cough, and vomiting; soreness of the eyes with a discharge had appeared 2 days before. On admission to hospital his temperature was 103° F., pulse rate 100, and respiration rate 30. He had (1) bilateral conjunctivitis with vesicles on the conjunctiva and a mucopurulent discharge; (2) ulceration of the nares and pustules on the upper lip; (3) discrete adherent white patches up to ¼ inch in diameter on the inner sides of both cheeks and on the tonsils; and (4) numerous rhonchi over both lungs. A

throat swab showed "no haemolytic streptococci, no *Corynebacterium diphtheriae*, no Vincent's organisms"; the blood count was normal and the Wassermann and Kahn reactions negative. Two days later he developed (5) a purulent discharge and (6) vesicles (containing a pale sterile fluid free from cells) on an erythematous base on the dorsum of the arms; after 2 days the vesicles had spread to the lower limbs and trunk. They varied in size and rapidly developed dark crusts. The temperature ranged from 102° to 104° F. and the fever did not respond to sulphamezathine, so 6 days after admission penicillin, 15,000 units intramuscularly every 3 hours, was given and the condition improved. The corneae remained clear; irrigations with boric acid lotion and eye drops of 10% "albucid" (sulphacetamide) every 2 hours were prescribed. A fortnight after admission the patient developed for 2 days slight consolidation at the right base with a pleuritic rub, cough, and viscid sputum. A throat swab at this time grew *Staphylococcus aureus*; biopsy of a vesicular lesion showed "slight vascular engorgement of the corium and a little oedema of the Malpighian cells"; blood culture was sterile. Three weeks after admission the eyes were clear, the skin lesions were only brown discoloured patches, and 10 days later the mouth was clear and the urethral discharge had ceased, but the prepuce was so densely adherent to the glans penis that circumcision was performed.

Henry Cohen.

A Report of an Epidemic of Mild Lymphocytic Meningitis in Burma. KIBBE, F. W., and BREADENKOPF, W. G. (1946). *Johns Hopk. Hosp. Bull.*, 79, 365.

Twenty-three cases of mild lymphocytic meningitis are reported; they probably formed about half those occurring in an epidemic among units served by a U.S. general hospital in Burma. The symptoms were: severe bilateral bursting frontal headache; "eye-ache" and pain on moving the eyes, fatigue, weakness, and nausea; vomiting; inconstant prodromal symptoms; fever (100° to 103° F.); sweating; conjunctival congestion; tremor; and neck rigidity, but not so marked as in bacterial meningitis. Reflexes, ocular muscles sensation, and mentality were normal, and there were no convulsions. Eight cases showed moderate lymphocytosis, the greatest shift being from 12% to 44%. There was no eosinophilia. Four patients while in hospital showed a probably significant heterophil antibody agglutination, and of 20 readmitted cases, seen 2 months after the onset, 13 gave agglutination titres of 1 in 7 to 1 in 224. Complement-fixation and virus neutralization tests for

lymphocytic choriomeningitis, mumps, dengue, and three types of encephalitis were negative. The cerebro-spinal fluid was abnormal in all cases. Cell counts varied from 0 to 490 per c.mm., lymphocytes predominating except in the case with the highest count. Protein varied from 29 to 70 mg. per 100 ml. There was no relation between these findings and the severity of the symptoms, and in 4 cases the fluid was normal 4 weeks later. There was no evidence of spinal block, though manometric pressure determination could not be made.

The infection is regarded as being due to a virus, in the absence of bacterial, spirochaetal, or chemical factors, or of an associated disease likely to produce a reactive meningitis. The frequency of the heterophil reaction suggests infectious mononucleosis, some features of which were present. The test is, however, apparently non-specific, and a number of epidemic fevers in Japan give a positive Paul–Bunnell reaction. Poliomyelitis is unlikely, and tests for types of encephalitis were negative. The dengue group of diseases also appeared unlikely to be implicated. The absence of neurological dysfunction makes lymphocytic choriomeningitis again improbable. The disease is accordingly classed among the group of benign meningitides named "acute aseptic meningitis," in only one-third of which have specific viruses been found. If any virus were isolated or identified it would probably fall into the dengue group.   *W. A. Bourne.*

An Outbreak of Trichinosis in New York City with Special Reference to the Intradermal and Precipitin Tests. SHOOKHOFF, H. B., BIRNKRANT, W. B., and GREENBERG, M. (1946). *Amer. J. publ. Hlth.*, 36, 1403.

The consumption of pork products from a single wholesaler was held responsible for an outbreak of trichiniasis, involving 84 cases, occurring in New York in the early part of 1945. The values of the precipitin and intradermal tests as aids to diagnosis during the actual illness are considered. Fifty out of the 84 of the sufferers had eaten a sausage preparation called mettwurst. None of the pork products consumed had been subjected to the usual process of freezing for 20 days at 5° F.

The incubation period varied between 2 and 35 days, but was usually between 5 and 17 days. Attention is drawn to the fact that in this, as in most of the large-scale outbreaks of this disease in recent years, there were no fatalities. The main symptoms were oedema of the eyelids, muscle pains, and fever. Gastro-enteritis, generally considered an early condition, was present in only 7 cases. Differential blood counts were carried out in 72 cases; in 64 eosinophils were found to exceed 10% of the total white cells, and in only 1 of the remaining 8 was the eosinophil count less than 5%. The eosinophil count is considered the most useful single laboratory test in the early diagnosis of trichiniasis, and if eosinophilia is not present the diagnosis should be doubted unless the infecting organisms are isolated.

The precipitin reaction was performed in 40 cases and gave a positive result in 33, with 5 doubtful positive reactions. The intradermal reaction with 1 in 10,000 dilution of trichinella antigen was performed in 21 of the 84 cases and gave a positive result in 15 in some after repeated tests. The earliest positive reaction was obtained on the sixth day of illness, but the best results were achieved after the end of the second week, which makes the test somewhat limited in its use. The authors question the generally accepted view that the skin test becomes positive before the precipitin test, as their results indicate

the reverse. The advisability of using both tests in all doubtful cases is suggested. · Muscle biopsies were not carried out because it was considered that the other available tests gave positive reactions earlier in the illness.   *H. C. Maurice Williams.*

False Positive Kahn Reactions. Loss of Titer on Storage of Serum in Ice Box. LUBITZ, J. M. (1946). *Amer. J. clin. Path.*, 16, 768.

It had been noted that sera from patients after smallpox vaccination which gave false positive Kahn reactions tended to become negative when exposed to ordinary room temperatures for several days. The author tested the sera of 59 syphilitic patients (92 sera and 219 tests) over a period of 2 to 6 months with the quantitative Kahn test; the sera were stored at $+2°$ to $+4°$ C. Of the 92 sera, 25 (27·1%) showed a moderate loss of titre, 10 (10·9%) showed a gain, and 57 (62·0%) maintained a constant titre. He also tested two groups of false positive sera. Group I consisted of 13 patients with smallpox, 15 with malaria, 5 with upper respiratory infections, 2 with scarlet fever, and 1 each with tuberculosis of bone, infectious mononucleosis, gonococcal arthritis, lymphogranuloma venereum, and a "general biologic reaction"; 109 sera were taken and 208 tests carried out; all showed a decrease of titre or became negative at various periods, usually within a month. Group II consisted of 1 patient with pinta whose serum maintained its titre for 3 months, and 5 with leprosy; 2 sera maintained and 4 lost titre. Illustrative cases with results of serum tests are given in seven tables. That the serum from the patient with pinta maintained its titre is not considered surprising, since the disease is due to a spirochaete; the reactions of the leper sera are not so easily explained. The loss of titre in general may be due to non-specific antibody being more labile, or to an inhibitor, present in the serum, becoming activated: the difference in behaviour of syphilitic and non-syphilitic sera suggests that the two types of antibody are not the same.   *T. E. Osmond.*

Comparison of the *in Vitro* Antigonococcal Actions of Penicillins G, F, K, and X. NELL, E. E., and HILL, J. H. (1947). *Amer. J. Syph.*, 31, 14.

In this comparison the *in vitro* activity of penicillin G and X (both pure crystalline) and of F and K (approximately 90% pure) has been tested against freshly isolated cultures of gonococci obtained from a large number of patients. The authors are doubtful whether the gono-coccus is an appropriate organism for standard testing and consider that the meningococcus might be more suitable. The tests indicate that pure crystalline penicillin is 7·3 times more efficient *in vitro* than is pure crystalline G against freshly isolated gonococci from 76 different patients, and that 90% pure penicillin is 3·3 times as efficient against the same organisms. Pure crystalline G and 90% pure K were also tested against gonococci from 40 different sources, and it was shown that the average *in vitro* efficacy of K was 1·5 times that of G.

[It will be appreciated that the fate of K in the body would discount its use as a practical therapeutic agent. The authors rightly urge that further clinical trials of crystalline penicillin X in gonorrhoea should be made as soon as possible. Their technique is ingenious and the paper should be read in the original by those who are interested.]   *G. L. M. McElligott.*

BIOCHEMISTRY

Absorption and Excretion of Water. The Antidiuretic Hormone. VERNEY, E. B. (1946). *Lancet*, 2, 739.

In the dog the peak of the diuresis which follows the ingestion of 250 ml. of water occurs after 50 minutes, but the absorption curve reaches the 250-ml. level at $35\frac{1}{4}$ minutes. Subtraction of the water-excretion curve from the absorption curve gives the water load curve, which represents the amount of water temporarily held in the tissues in excess of the optimal amount. The peak of the water-load curve occurs 15 minutes earlier than that of the excretion curve. There is therefore a delay of 15 minutes between the maximal stimulus to excretion and the maximal response of the kidney to the stimulus. The diuresis is inhibited by muscular exercise, emotional stress, or a rise in the osmotic pressure of the carotid arterial plasma.

The inhibitory effect of exercise is accompanied by an increase in the chloride, nitrogen, and pigment content of the urine. This inhibitory effect is only apparent and is really due to emotional stress, as emotional stress produced by mild faradism without exercise gives rise to a similar inhibition, which might be due to vasomotor changes, though the rise in chloride excretion is against such an interpretation. This hypothesis is disproved by the fact that the inhibitory response is unaltered by complete denervation of the kidneys. The argument that the inhibition may be due to release of adrenaline is disproved by the fact that the response is not altered by the removal of one adrenal gland and the denervation of the other. Moreover, occlusion of the renal artery, whether complete or partial, causes a different response, the inhibition of urinary flow rising suddenly as soon as the compression ceases. Lastly, direct measurement of renal blood flow during emotional stress demonstrates the independence of the response.

Comparison of the inhibitory response to emotional stress with that due to the intravenous injection of adrenaline and of posterior pituitary extracts shows significant differences. Adrenaline produces a sudden fall in urinary excretion and an almost equally sudden recovery, a response unlike that of emotional stress. On the other hand, a suitable dose of posterior pituitary extract produces a response identical with that of emotional stress. There is a latent period (absent in experiments with adrenaline) followed not only by a decrease in urinary excretion but by an increase in urinary chloride and nitrogen. The return to normal is more gradual than after adrenaline injection. Exact similarity between the responses to emotional stress and to injection of posterior pituitary extract does not prove a causal identity. The proof is found in the fact that the polyuria which follows removal of the posterior pituitary subsides after a week. Thereafter emotional stress fails to elicit the typical response, which, however, occurs as usual after the injection of posterior pituitary extract.

Increased activity of the sympathetic nervous system may overcome the inhibitory effect of the posterior pituitary. The action of adrenaline is not specific, tyramine being as effective. It is probable that increase of cerebral blood flow is the common factor preventing release of the antidiuretic substances. It seems, therefore, that low sympathetic tone associated with noxious stimuli is favourable to the prolonged secretion of antidiuretic substance. The release of this substance is caused by such mild disturbances of the central nervous system that it becomes important to determine whether the secretion is varying continuously under the control of some factor in the animal's internal environment the maintenance of which within a narrow physiological range is important for the animal's welfare. It seemed probable that this factor is the osmotic pressure of the carotid plasma, as shown by the finding that the injection of hypertonic solutions into the carotid artery causes an inhibition of urinary flow, the magnitude of the response varying with the tonicity of the solution at constant rate and period of injection and with the period of injection at constant volume and tonicity. This effect disappears after removal of the posterior pituitary but can be mimicked by the injections of posterior pituitary extract. It can be produced by the injection either of sodium chloride or of iso-osmotic dextrose, and is therefore dependent on the osmotic pressure and not specifically the substance. Comparison with the effects of posterior pituitary injections demonstrates that increase in blood chloride of 8 mg. per 100 ml. causes the pituitary to secrete 1 μU. per second. There can be little doubt that the secretion of the posterior pituitary is a hormone in the true sense, its liberation being continuously governed by the contemporary concentration of chloride and possibly of other osmotically active substances in the arterial plasma. The control is presumably effected by osmoreceptors connected by nervous paths with the pituitary.

[This important paper should be read in full.]

Raymond Greene.

Biochemistry of Inflammation. MENKIN, V. (1947). *Lancet*, 1, 660.

The author believes that the clinical features of acute inflammation are due to the liberation of "biochemical substances" from injured cells. In this paper he summarizes his extensive work on this subject and describes the five substances he has so far isolated from inflammatory exudates.

Leukotaxine increases capillary permeability, so that plasma proteins, dyes, colloids, and bacteria readily escape into and are concentrated in the injured area. The author has isolated this in a relatively pure form; it seems chemically not to be a histamine-like substance, as postulated by Lewis, but a relatively simple polypeptide. It is positively chemotactic to polymorphonuclears, but does not induce general leucocytosis. Leukotaxine is abundant in the rabbit's succus entericus, and may be found in such fluids as the contents of ovarian cysts.

L.P.F., a leucocytosis-promoting factor contained in inflammatory exudates, causes leucocytosis and hyperplasia of marrow granulocytes and megakaryocytes. The author has also isolated this substance, which appears to be a pseudoglobulin and not present in normal serum.

Necrosin is associated with the euglobulin fraction of inflammatory exudates but not of normal serum. Intradermal injection of necrosin causes an intense inflammatory reaction followed by superficial necrosis; the lymphatics are occluded by fibrin, and blood vessels may be thrombosed. Intravenous injection may cause focal necrosis of the liver, pleural effusion, intestinal haemorrhages, or renal changes. Necrosin is lethal to mice.

Pyrexin is another factor in the euglobulin fraction of inflammatory exudates which induces fever when injected intravenously into mice. Pure necrosin is not pyrogenic. Injection of the whole euglobulin fraction of inflammatory exudates causes leucopenia as well as necrosis and pyrexia. Vomiting, diarrhoea, and "general apathy" may also occur. The leucopenia seems to be

due to "a trapping of the leucocytes in lungs, liver, and spleen." It is ascribed to a specific *leucopenic factor*.

[This paper gives little idea of the stimulating qualities of Menkin's book, *The Dynamics of Inflammation*. The author's postulate, that the effects he describes in this paper are due to definite chemical entities constantly present as such in inflammatory exudates, may not gain general acceptance until much confirmatory evidence is available.]　　　　　　　　　　　　　*Martin Hynes.*

Urinary Excretion of Phosphatases in Man. BURGEN, A. S. V. (1947). *Lancet*, 1, 329.

Urinary phosphatase was determined by the hydrolysis of monophenyl phosphate and the estimation of liberated phenol by the diazo method of Theis and Benedict, which is unaffected by uric acid. The daily excretion of phosphatases in the urine of 50 males and 25 females without genito-urinary disease was estimated. Alkaline-phosphatase excretion was low and irregular and showed no significant variation with age or sex. The average daily acid-phosphatase excretion in females is about 50 units, irrespective of age. The male acid-phosphatase excretion level is similar in childhood, but rises sharply at puberty above that of females, reaching a maximum of 350 units between 30 and 40 years and returning to the prepubertial level in old age. The normal rate of haemolysis will explain female urinary excretion of acid phosphatase, but the acid-phosphatase excretion increase in the male during the reproductive period is usually assumed to come from the prostate. Samples collected over 24 hours from 3 men by suprapubic cystotomy suggest, however, that part of the excess acid phosphatase is excreted through the kidney. No alteration in phosphatase excretion was found in disease of the prostate or in nephritis.　　　　　　　*E. F. McCarthy.*

The Nicotinamide Saturation Test. ELLINGER, P., and HARDWICK, S. W. (1947). *Brit. med. J.*, 1, 672.

Urine samples collected every 24 hours for 11 days from 1 diabetic and 5 healthy persons were examined for nicotinamide methochloride by Coulson, Ellinger, and Holden's method (*Biochem. J.*, 1944, 38, 150). The subjects were given oral doses of 100 mg. of nicotinamide after the third and ninth urine collection, and of 1 g. of methionine every 12 hours on the seventh to eleventh day. The methionine did not affect the methochloride excretion rate, but when the dosage of nicotinamide was prolonged so as to deplete the body stores of "methyl-donators," added methionine increased the urinary response. The effect was demonstrated by experiments on groups of 5, 8 (3 pellagrins), and 8 (3 pellagrins) mental patients, whose 24-hour urine samples were collected for 14 days. The patients received either oral or subcutaneous doses of nicotinamide after the third to the thirteenth urine collection and oral doses of 1 g. of methionine every 12 hours on the ninth, tenth, and eleventh days of the test. The effect of methionine was greater after a daily intake of 500 mg. of nicotinamide than after one of 100 mg.

The saturation test described by Ellinger, Benesch, and Hardwick (*Lancet*, 1945, 2, 197) would be affected by a depletion of the body reserves of "methyl-donators," and the following modified test is recommended. Twenty-four-hour urine samples are collected for 18 days and the urinary nicotinamide methochloride is estimated. Doses of nicotinamide (100 mg.) are administered subcutaneously after the third and twelfth, orally after the sixth, and rectally after the ninth urine collection, and the percentage of ingested nicotinamide excreted during each 24- and 72-hour period after dosing is calculated. The results obtained for 6 physically fit and 3 pellagrin mental patients are discussed. The urinary response to nicotinamide administered rectally was slightly less than to that given orally, showing that nicotinamide can be absorbed from the lower intestine.　　　－　*J. E. Page.*

Hydrogen Ion Concentration (pH) of Normal Vaginas. KARNAKY, K. J. (1947). *West. J. Surg. Obstet. Gynec.*, 55, 103.

The author used the Beckman pH meter and Rakoff's vaginal electrodes to study vaginal pH in 52 pregnant and 15 non-pregnant women. This report differs from previous ones in that the author has determined the pH in different sites of the vagina—namely, anterior fornix, posterior fornix, and left and right lateral walls, the averages for these being 4.26, 4.37, 4.34, and 4.46 respectively. In non-pregnant women the average was 4.34, while in pregnancy the figure was 4.38. The over-all pH average in apparently normal vaginas varies between 3.27 and 4.99.　　　　　　　　　*C. W. Kimbell.*

Use of a Tourniquet to Prolong the Effect of Penicillin. CARLINFANTI, E., and MORRA, F. (1947). *Lancet*, 1, 521.

The rate of absorption of penicillin injected subcutaneously into thigh or arm can be considerably slowed by exerting light pressure with a rubber tourniquet proximal to the site of injection. Results comparable to those obtained with oil-beeswax preparations can thus be achieved. The penicillin concentrations in the blood were estimated by the capillary-tube method of Fleming and by another method devised in St. Mary's Hospital, London, with phenol-red as an indicator of the growth of staphylococci. Trials showed that the tourniquet should be applied for times varying from 1 hour after injections of 10,000 units to 5 hours after 100,000 units. With this method of administration the number of injections can be reduced to three of 75,000 units in 24 hours or to five of 100,000 units in 48 hours without letting the penicillin level fall below the lowest effective one.　　　　　　　　　　　*Geoffrey McComas.*

Estimation of Penicillin in Serum. Use of Glucose, Phenol Red, and Serum Water. FLEMING, A., and SMITH, C. (1947). *Lancet*, 1, 401.

A micro-method is described for the estimation of penicillin in serum; it is a modification of previous methods described by Fleming and by Dr. J. Fielding. The medium used consists of serum (human, horse, sheep, or ox) 2 ml., 10% glucose solution 2 ml., water 6 ml., and phenol red, saturated solution, 0.25 ml. This can be sterilized either by steaming in bulk or by boiling for a few minutes. A suitable quantity of the medium is inoculated with the test organism (5 ml. of a 24-hour broth culture to 1 ml. medium); the authors use a haemolytic streptococcus, which produces acid more quickly than staphylococcus. Serial dilutions of the serum to be tested (25-ml. volumes) are made in the inoculated medium on the surface of a paraffined slide. Glass capillaries made from soda glass, not from hard

glass, are taken, and each of the drops is touched with the end of a capillary tube so that the fluid runs into it. The tube is sealed (this can be omitted) and placed horizontally on a "plasticine"-covered slide and incubated. Tubes in which the streptococci have grown are bright yellow and show a heavy precipitate; tubes in which growth has been prevented by penicillin are red or red-violet. The test can also be done as a macro-method in small test-tubes. The authors have found this method rapid and convenient; they discuss the reasons for adopting this procedure and the permissible modifications.

[Presumably the number of units of penicillin present is read by comparison with a standard set of tubes containing known concentrations of penicillin.]

F. Hawking.

17-Ketosteroids in the Diagnosis of Adrenal Tumors. JOHNSON, H. T., and NESBIT, R. M. (1947). *Surgery*, 21, 184.

Urinary 17-ketosteroids can be divided into *alpha* and *beta* fractions, the *alpha* produced by the adrenal cortex and testes, the *beta* by adrenal cortex only. The authors report 3 cases of adrenal cortical carcinoma with markedly elevated total 17-ketosteroids in which differentiation into *alpha* and *beta* fractions was of value. The first case was in a woman of 31 years with menstrual disturbances, gain in weight, hypertrichosis, and a mass in the right upper quadrant of the abdomen. Total urinary 17-ketosteroids amounted to 75 mg. per day (*alpha* 19.7, *beta* 55.3). (Normal for adult women: total 2.7 to 8.1; 5 to 15% *beta*.) A carcinoma of the right suprarenal was removed. Ten days after operation total 17-ketosteroids measured 3.8 mg. per day. The patient was well one year after operation. The second case was in a 2-year-old girl with pubic hair and a large clitoris. Excretory urograms showed a right adrenal tumour depressing the right kidney. Urinary 17-ketosteroids measured 78.5 mg. per day (*alpha* 15.7, *beta* 62.3). (Normal for a child aged 4 to 7 years: average of 1.3 mg. per day.) An adrenal cortical adenoma undergoing carcinomatous change was removed from the right side. Ten days after operation 17-ketosteroids measured 0.9 mg. per day. The child was well 6 months after operation, but there was only slight retrogression of pubic hair. The third case was in a man of 43 with an inoperable carcinomatous mass in the left adrenal region and no hormonal symptoms; 17-ketosteroids amounted to 86 mg. per day (*alpha* 36.6, *beta* 49.4). (Normal for adult man: 3.4 to 15 mg. per day.) The amount fell with radiotherapy, which produced clinical improvement, but rose again with re-growth of the tumour. The patient was discharged as unfit for further treatment.

D. H. Patey.

Mechanism of Hydrogen Peroxide Formation by Spermatozoa and the Role of Amino-acids in Sperm Motility. TOSIC, J. (1947). *Nature, Lond.*, 159, 544.

Bull spermatozoa lose respiratory activity and motility when kept under aerobic conditions in egg-yolk medium. This inhibition is due to hydrogen peroxide, which is formed by enzymic action for some dialysable constituent of the egg yolk. The chemical behaviour of this constituent during attempts at its isolation suggested that it is an amino-acid. *l*-Tryptophane, *l*-tyrosine, and *l*-phenylalanine can replace egg-yolk medium and in the

presence of living spermatozoa give rise to hydrogen peroxide. Only these three amino-acids of the many tested had this property. Such specificity of an *l*-amino-acid-oxidase is unusual. The adverse effects of hydrogen peroxide on sperm respiration and motility are produced with concentrations that are chemically undetectable, but appreciable since the effect is abolished by the addition of catalase.

[The finding will be useful in arranging optimal conditions for the storage and transport of sperm for artificial insemination, but probably has no physiological importance. Judgment on this will await more detailed description of the aerobic conditions needed for the enzymic activity.]

P. C. Williams.

Determination of Sex Hormones by the *m*-Dinitrobenzol Reaction. (Bestimmung von Keimdrüsenhormonen mittels der *m*-Dinitrobenzolreaktion.) ZIMMERMAN, W. (1946). *Schweiz. med. Wschr.*, 76, 805.

This is a review of the dinitrobenzene assay of sex hormones and its applications in physiological, biochemical, bacteriological, and clinical research. The author's method for the assay of sex hormones in urine and blood is given in detail. The method has also been worked out for the assay of androgenic hormones in blood.

M. B. Klein.

HAEMATOLOGY

Genetic and Constitutional Causes of Foetal and Neonatal Morbidity. LEVINE, P. (1946). *Ann. N.Y. Acad. Sci.*, 46, 939.

In some 92% of cases of erythroblastosis foetalis the mother is Rh-negative—that is, all her cells are not agglutinated by the anti-Rh$_0$ (anti-D) serum and her serum contains anti-D agglutinins or blocking antibodies. In about 8% of cases of erythroblastosis foetalis the mother is Rh-positive; in these cases the factor responsible is one of the other Rh antigens, such as Hr[c] or in some cases the ordinary A and B antigens or possibly other rare blood group factors. When A or B are responsible " tests of the affected infant's saliva or the deceased infant's organs should reveal the infant to belong to the non-secretor type." "The demonstration of specific increase in anti-A or anti-B agglutinins is contributory evidence." [These two statements are difficult to reconcile, since it has been shown by Smith that iso-immune responses to A and B during pregnancy only occur when the infant is a "secretor."] The statistical proof of the part played by A and B in causing occasional cases of erythroblastosis foetalis is given in the following table:

Matings	Compatible %	Incompatible %
Random	65	35
215 Rh-negative mothers*	75	25
28 Rh-positive mothers*	50	50

*Mothers of erythroblastotic infants.

In this table incompatible matings are defined as those in which the father's blood contains A or B and the mother's serum contains corresponding anti-A or

anti-B agglutinins. The higher value for incompatible matings in the Rh-positive mothers indicates that iso-immunization by the A or B factors can explain at least some of the exceptional cases. That incompatible matings of this kind also sometimes cause early foetal death is suggested by the following figures:

Matings	Compatible	Incompatible
115 with 2 or more mis-carriages	46%	54%
43 with 2 miscarriages or stillbirths	44%	56%

" These figures are highly suggestive and probably significant." Obviously the statistical proof cannot be expected to be so convincing as in the case of the connexion between the Rh factor and erythroblastosis foetalis, since there are certainly many causes of abortion and stillbirth besides iso-immunization to A and B. Further support for the view that immunization against A and B occasionally causes foetal death is provided by the observation that the proportion of A children in matings between A fathers and O mothers is slightly smaller than that produced by matings between O fathers and A mothers. Erythroblastosis foetalis occurs very rarely in the first-born; however, transfusion of Rh-negative women at any time before pregnancy tends to increase this occurrence, especially in its more severe form.

Erythroblastosis Foetalis in the First-born of Rh-negative Women

		Transfusion History	
		Previously Transfused	Never Transfused
Severity of disease:			
Mild		1	4
Severe		5	4
Foetal deaths		10	1
Total		16	9

All persons giving transfusions of blood should be aware of this most serious risk.

[This paper is a summary of information previously published, but it is worth reading even by those who are already familiar with Levine's contributions. Only a few points in it can be referred to in this abstract.]

P. L. Mollison.

Isolation and Purification of Blood Group A and B Substances ; Their Use in Conditioning Universal Donor Blood, in Neutralizing Anti-Rh Sera, and in the Production of Potent Grouping Sera. WITEBSKY, E. (1946). *Ann. N.Y. Acad. Sci.*, 46, 887.

At present two different preparations of blood-group-specific substances are available commercially in the U.S.A.: (1) the A specific substance isolated from hog stomach, and (2) the AB specific substance isolated from horse stomach. Both are chemically free from protein, and neither will sensitize guinea-pigs. These purified preparations are of practical use:

1. In neutralizing the anti-A and anti-B iso-antibodies of blood of group O. There is now abundant evidence that anti-A and anti-B agglutinins in group O blood frequently cause some degree of destruction of the recipient's erythrocytes when the latter are of group A or B; occasionally the degree of destruction is sufficient to cause severe or even fatal reactions, a danger that can be avoided by the addition to the blood of small amounts of AB substance to reduce the strength of the antibodies to a harmless level.

2. In suppressing anti-A and anti-B iso-agglutinins in anti-Rh sera. In making Rh tests, sera obtained from human beings who have been sensitized by pregnancy or transfusion are most frequently used. These of course often contain anti-A or anti-B agglutinins, which must be removed or neutralized before the sera can be used to test AB cells. Complete suppression of the anti-A and anti-B agglutinins can be effected by quite small amounts of the purified group-specific substance.

3. In production of potent grouping sera. As little as 0·1 mg. of blood-group-specific substance injected intravenously into human beings will often produce a tenfold to one-hundred-fold increase in the original iso-agglutinin titre. At the same time the avidity of the sera is increased (a minority show a small or negligible response). *P. L. Mollison.*

The Clinical Significance of Rh Antibodies (Rh Agglutinins and Blocking Antibodies) in the Sera of Rh-negative Mothers ; A Study of 179 Cases. HOWARD, J., LUCIA, S. P., HUNT, M. L., and McIVOR, B. C. (1947). *Amer. J. Obstet. Gynec.*, 53, 569.

In an effort to correlate the laboratory findings with the clinical condition of the babies at birth, the authors studied the antibody titre of 179 Rh-negative expectant mothers. The maternal sera were examined for complete and incomplete antibodies. The investigations were carried out in 1944–5, and the latter type of antibody was detected by its blocking activity with Rh_0 (cDe/cde or cDe/cDe) cells and not by the Race-Coombs test. The 179 pregnancies are divided into four groups: group 1, normal Rh-negative children; group 2, normal Rh-positive children; group 3, "subclinical" haemolytic disease of the newborn; group 4, severe haemolytic disease of the newborn. In group 1 (61 cases), only 9 showed isolated complete anti-Rh antibodies, probably carried over from previous pregnancies. Group 2 (78 cases) showed no ante-partum antibody production, but a small titre of either complete or incomplete antibody might appear post partum, reaching a maximum usually in the second week. There was no significant difference between primiparous and multiparous women. Group 3 (17 cases) showed production of antibodies in the last 12 weeks of pregnancy. Ante partum, in this group, there appeared to be a predominance of blocking antibody, but the amount of this fell after delivery. Of the 17 mothers, 3 were primiparous and 14 multiparous —a significant difference. In group 4 (20 cases) antibodies were always demonstrated ante partum and usually the amounts of the two varieties (complete and incomplete) varied inversely; in no case did they rise or fall together. Most commonly the complete antibodies diminished as pregnancy advanced and the incomplete antibodies increased.

In 4 cases stillborn children were delivered, and in 3 of these complete antibodies were demonstrated in the maternal serum. On the other hand, in only 3 of 12 infants born alive were blocking antibodies not demonstrated in the mother's blood. The authors point out that this contradicts Wiener's view that the incomplete antibody is of more serious import, and discuss the possibility that the incomplete antibody acts as a protective substance for the foetal red cells. The antibody titres in group 4 rose to a maximum about the second week post partum and then declined.   *A. J. Buller.*

The Transition Forms of Blood Groups. HIRSZFELD, L. (1947). *J. Immunol.*, 55, 141.

A review of the author's theory of the antigenic structure of the A_1 A_2 BO blood groups is presented. By parallel titrations of red cells of group A_1 against anti-O (goat anti-Shiga bacillus) and anti-A sera, several different forms can be separated. Some appear to have more O substance and less A substance and some the reverse, the total antigenic substance being constant. The reaction of A blood with anti-O serum does not depend on the blood being heterozygous O, for in testing a large number of samples of blood, many of which must have been A_1A_1 (18% in Poland), none was found which was completely negative with the anti-O serum. Further, the blood of known A_1A_1, A_1A_2, A_1A_2, and A_2B persons was agglutinated by the anti-O serum. The author therefore considers that the cause of the agglutinability "must be searched for in the structure of the A and B genes as such."

. The hypothesis is put forward that there is a range of increasingly complete mutations stretching from O through $A_5{\rightarrow}A_4{\rightarrow}A_3{\rightarrow}A_2{\rightarrow}A_m{\rightarrow}A_r{\rightarrow}A_1$ to A_c. A_5 is so weak in A that the cold agglutinin anti-A is present in the serum; A_4 is what is usually thought to be a group O with anti-A missing from its serum. The stages A_m, A_r, and A_1 correspond to the classical A_1 gene, and the hypothetical A_c is completely A. The mutations appear to be changes of similar nature, but of increasing extent. A parallel series is proposed from O to B_c. People who possess anti-O are considered to be A_cA_c, A_cB_c, or B_cB_c. The rarity of persons of these groups explains the infrequency of anti-O.

[It is a pity that this important paper is not more detailed; the conceptions of gene and genotype are not clearly distinguished, and from reading this paper alone one would be left wondering whether the bloods classified as A_m, A_r, and A_1 were known AO heterozygotes, for, if they were not, it seems that A_mA_m might have as much A and O substance as A_1O, and be indistinguishable from it. This aspect is considered in *Ann. Inst. Pasteur*, 1940, 65, 251 and 386. The author suggests that the direction of mutation is away from O towards A_c, and that O may eventually disappear. Such progressive evolution is not in accord with current doctrine. It also conflicts with Ford's view that the blood groups represent a balanced polymorphism (*Genetics for Medical Students*, 1942, p. 19). The evolutionary conjectures are not, however, as the author points out, an essential part of his theory. The theory does not seem capable of explaining the presence of α_1 in the serum of A_2B persons when this α_1 is active at body temperature. At least three such cases have been reported by experienced workers.]   *R. R. Race.*

Substitution Transfusion : A New Treatment for Severe Erythroblastosis Fetalis. WALLERSTEIN, H. *Amer. J. Dis. Child.*, 73, 19.

A method is described of substitution transfusion given immediately after birth in the sub-icteric stage of haemolytic disease of the newborn to prevent subsequent damage to the liver and brain. Hepatic damage in cases of icterus gravis by excessive red-cell haemolysis and a reactive liver haemopoiesis has been recognized. If this is to be avoided the end-products of haemolysis must be removed or their formation prevented. This may be done by withdrawal of the infant's Rh-positive cells and their replacement by harmless Rh-negative cells. Withdrawal and replacement should be carried out simultaneously. The technique is as follows: (1) Isotonic saline or pooled plasma is infused into an arm vein. (2) Blood (50 to 60 ml.) is withdrawn by syringe from the longitudinal sinus. (3) Rh-negative blood of a compatible group replaces the plasma or saline infusion. (4) When an estimated substitution of 80% has been accomplished the needle is removed from the fontanelle. The estimate is based on the infant's weight, counting the total blood volume as 10%. (5) The infusion is continued until 75 to 100 ml. of Rh-negative blood has been given in excess of the amount recovered, 10 ml. of 10% calcium gluconate being injected into the vein to counteract the citrate in the blood or plasma. The procedure takes from $\frac{1}{2}$ to $1\frac{1}{2}$ hours. Alternatively blood may be withdrawn from a small branch of the radial artery at the wrist. It is difficult to obtain enough blood from superficial veins. [Withdrawal of blood from the longitudinal sinus is not without its dangers in a jaundiced baby.]

Details of cases in which the prognosis was considered grave are given. Seven patients recovered and 2 died, both of whom might have recovered if treatment could have been started in the first 24 hours of life. Emphasis on anaemia as the indication for treatment is an error; the protection of the liver is the primary indication. About 30% of cases of haemolytic disease show liver damage and 10% kernicterus; until these changes can be accurately forecast, substitution transfusion should be used in all cases. If cases are to be selected the following are the most helpful criteria: (1) multiparity with a history of erythroblastosis in previous pregnancies; (2) serological studies; (3) during delivery, the presence of icteric amniotic fluid; (4) an excessively large, pale placenta; (5) excess of nucleated red cells in the blood in the cord. With such indications preparation should be made in advance and substitution therapy instituted as soon as the baby is born.
          A. G. Watkins.

The Treatment of Lymphoblastic Leukemia with Crude Myelokentric Acid. MILLER, F. R., HERBUT, P. A., and JONES, H. W. (1947). *Blood*, 2, 15.

Miller, in association with other workers, has previously shown that myelokentric acid and lymphokentric acid are present in varying amounts in the urine of patients with acute and chronic leukaemia, Hodgkin's disease, and lymphosarcoma. He has suggested that these substances are of fundamental importance in the abnormal processes of blood-cell production in the leukaemias, since they may constitute a balance mechanism in normal blood-cell proliferation and maturation. Lymphokentric acid is thought to bring

about lymphoid proliferation without maturation, while myelokentric acid by inhibiting proliferation of lymphoid cells allows them to mature. Maturation of myeloid cells is brought about by the action of lymphokentric acid, which inhibits their proliferation. The present paper discusses the effect of treatment of 8 cases of lymphoblastic leukaemia with myelokentric acid recovered from the urine or faeces, since on Miller's hypothesis there is a deficiency of this material in this condition. The authors claim 13 partial remissions. [Since no patient lived longer than 8 months the significance of the results is doubtful.]   *Janet Vaughan.*

Comparison of the Effects of Massive Blood Transfusions and of Liver Extract in Pernicious Anemia. DAVIDSON, C. S., MURPHY, J. C., WATSON, R. J., and CASTLE, W. B. (1946). *J. clin. Invest.*, 25, 858.

Five patients with Addisonian pernicious anaemia were given rapidly repeated blood transfusions until the erythrocyte counts and haemoglobin values in three were within normal limits. The symptoms attributable to the diminished oxygen-carrying capacity of the blood were relieved, but not such complaints as anorexia, apathy, and other digestive disturbances; these disappeared with liver extract. This sequence is typical of the nutritional deficiency disorders and indicates that blood transfusion does not relieve the fundamental defect in pernicious anaemia.

Injection of liver extract into the patients with normal blood counts following transfusion failed to cause any reticulocytosis, thus showing that this phenomenon occurs only when the stimulus of anoxia is exerted on the bone marrow. After transfusion but before the administration of liver the "bone marrow megaloblasts characteristic of pernicious anaemia" disappeared. The authors believe that a "maturation arrest" due to nutritional deficiency exists in pernicious anaemia. That haemolysis may be a factor of importance in the causation of anaemia they do not, however, attempt to deny.

[From the context it seems probable that the authors mean by the word "megaloblast" the most primitive nucleated red blood cell and not a series of abnormal elements. It is in this latter sense that most haematologists now employ the term, and as it is customary to regard the bone marrow in pernicious anaemia as dysplastic, the "maturation arrest" hypothesis becomes untenable.]   *R. Bodley Scott.*

Studies in Iron Transportation and Metabolism. V. Utilization of Intravenously Injected Radioactive Iron for Hemoglobin Synthesis, and an Evaluation of the Radioactive Iron Method for Studying Iron Absorption. DUBACH, R., MOORE, C. V., and MINNICH, V. (1946). *J. Lab. clin. Med.*, 31, 1201.

The authors have studied by Hahn's technique the rate at which small doses of radioactive iron injected intravenously are used for haemoglobin synthesis. In healthy adult men utilization is almost complete 75 days after injection ; normal dogs, however, used only 35 to 70% of the injected isotope. In iron-deficient patients and dogs there was complete utilization in 5 to 6 days. In hypoplastic anaemia less than 4% of the injected isotope was used; in pernicious anaemia in relapse utilization was insignificant until red-cell formation became active as a result of giving liver; it was then greatly accelerated and eventually became complete. In

haemolytic anaemia the percentage of an injected dose of radioactive iron in the peripheral blood varied greatly from time to time.

The authors seek to explain some of these results on the hypothesis that iron recently stored, whether derived from absorption from the alimentary tract, from injection, or from breakdown of haemoglobin, is retained in a form mobilized more easily than iron which has been stored for longer periods. It is thus used selectively for current needs. They point out that as radioactive iron is not used completely for haemoglobin synthesis it is unsafe to use as a measure of absorption the proportion of an oral dose appearing in the blood, as has been done in the past.   *R. Bodley Scott.*

Chemical, Clinical and Immunological Studies on the Products of Human Plasma Fractionation. XXXII and XXXIII. The Coagulation Defect in Hemophilia. An *in Vitro* and *in Vivo* Comparison of Normal and Hemophilic Whole Blood, Plasma and Derived Plasma Protein Fractions. LEWIS, J. H., DAVIDSON, C. S., MINOT, G. R., SOULIER, J. P., TAGNON, H. J., and TAYLOR, F. H. L. (1946). *J. clin. Invest.*, 25, 870, 876.

The ability of normal plasma to accelerate the coagulation of haemophilic blood has long been recognized. This effect has been shown to be unrelated to any of the formed elements of the blood. This paper records the fractionation of haemophilic plasma by the Physical Chemistry Department of the Harvard Medical School and subsequent testing of the fractions for anti-haemophilic activity in comparison with those of normal plasma. Both plasmas were similar in detectable protein content, Tiselius diagrams, and fibrinogen and prothrombin content. Normal plasma and normal fractions I, II, III, and IV-1 contain a substance capable of accelerating clotting of haemophilic blood. This substance is absent from haemophilic plasma and its fractions. The fractions mentioned contain most of the globulins and the fibrinogen.

Further efforts (article XXXIII, p. 876) have been made to determine the protein fractions of normal plasma which have the power of accelerating the clotting of haemophilic blood. After removal of fibrinogen from Fraction I by heat, the residual solution retains undiminished its anti-haemophilic power.

    R. Bodley Scott.

MORBID ANATOMY AND HISTOLOGY

The Evolution of Adenomas of the Large Intestine and their Relation to Carcinoma. HELWIG, E. B. (1947). *Surg. Gynec. Obstet.*, 84, 36.

A careful study at 1,460 consecutive necropsies disclosed that in 139 (9.5%) adenomata were present in the colon or rectum. In 80 cases the tumour was single, in 59 multiple. There was a steady increase in the incidence after the age of 30, the incidence reaching a maximum in the eighth decade, when 25.8% of the males and 20.5% of the females were affected. The author found that he could not classify the tumours on their macroscopical or microscopical appearances. There was no recognizable difference from the adenomata of familial adenomatosis coli. Different cross-sections of the same tumour showed varying microscopical appearances. The tumours were prone to trauma and infection, inflammation, ulcers, small abscesses, and haemato-

mata, haemosiderin being found in them at times. Evidence of previous or present colitis was found in only one case in association with an adenoma, and so the author considers colitis an unlikely exciting agent and believes the tumours to be true neoplasms.

It was noted that adenoma was commonest in the sigmoid colon, as was carcinoma. Early carcinomatous change was found in 10 of the 139 adenomata. This might occur in any part of the tumour—stalk, base, or periphery. In one case 2, and in another 3, adenomata showed early carcinomatous degeneration. Three of the 10 also had manifest carcinoma associated with the malignant adenoma. In the 1,460 necropsies 20 manifest or obvious carcinomata of the colon were found, and 13 of these had either benign adenoma or adenoma with malignant transition associated with them. Two cases in which a very early carcinoma appeared to be arising from the mucous membrane were also seen, both of which were unassociated with either adenoma or manifest carcinoma elsewhere in the colon. Thus a significant association between adenoma and carcinoma is shown, and the conclusion is drawn that, while some carcinomata arise directly from the mucous membrane, the majority arise in adenomata.

[This valuable article would repay study in the original.] *N. Tanner.*

Some Experiences with Bone Tumours. BRAILSFORD, J. F. (1947). *Brit. J. Radiol.*, 20, 129.

The author describes fully, with illustrations, a series of cases of bone lesions culled largely from his own observations. He describes a bone tumour in a young girl which, though radiologically diagnosed as a sarcoma, proved to be syphilitic. Here the onset 7 weeks after a blow at the site had suggested to the author the possibility of an infective lesion and caused him to advise against amputation. Another sarcoma-like tumour in the humerus of a child was accompanied by an apparently typical secondary deposit in the lungs. This precluded surgery, and sulphathiazole administration was advised. In 2 years, restitution of the bone to normal was complete and the "secondaries" had vanished. The author suggests that this may have been a manifestation of reticulo-endotheliosis. A case of sarcoma in the femur of a young man showed seemingly good response to radiotherapy, but there was local recurrence within a year, and later, after amputation, skeletal metastasis. The author suggests that surgery, to be successful, should follow closely on radiotherapy. A further case of bone disintegration occurred at the lower end of the femur in a boy, aged 14, who was suffering from paralytic deformities. This showed gradual spontaneous consolidation and recovery. The lesion is considered, for reasons based upon the radiological appearance, to have been probably a neurotrophic joint condition allied to Charcot's disease.

That biopsy may prove misleading, and should not in the face of contrary radiological evidence be given too much weight, is the moral drawn from 2 further cases of sarcoma: biopsy material was reported as inflammatory, but both cases developed secondary deposits. Two cases of osteoclastoma are described. The author states that radiotherapy may cure, but immobilization in plaster during the active stages is important. The first patient did well. The second was poorly immobilized, did not respond to treatment, and had eventually to undergo amputation for secondary haemorrhage. The patient is now otherwise well. Failure to give due weight to the

radiograph by the surgeon in the case of a man of 58 with a swelling at the knee-joint resulted in amputation. The lesion proved to be a Baker's cyst. The radiograph showed typical osteo-arthritic changes only. The rapid onset of secondaries after diagnosis of a bone sarcoma in another case described suggests the hopelessness of surgical intervention, but against this is quoted Speed's case in which, after amputation of a sarcoma, secondaries in the lung became shrunken and hard in outline, and remained unchanged 10 years later.

The author concludes that clinical and radiological evidence must be carefully correlated, that biopsy may be misleading, and that too hasty recourse to surgery may be ill-advised. He makes a plea for more emphasis on clinical radiology and less upon physics during the training of students. *A. M. Rackow.*

Tumors of the Testis. A Report on 922 Cases. FRIEDMAN, N. B., and MOORE, R. A. (1946). *Mil. Surg.*, 99, 573.

The authors report their study of 922 cases of testicular tumours collected at the Army Institute of Pathology (U.S.A.) between October, 1940, and May, 1946. Most of these occurred in men of military age, 18 to 38 years, although men up to 50 years of age were also included. The distribution of the tumours was: 35% seminomata, 35% teratocarcinomata (malignant teratoma), 19% embryonal carcinomata (including chorionic carcinomata), 4% adult teratomata, and 3% miscellaneous tumours. The incidence (corrected for army population in each age group) of teratocarcinoma was practically constant throughout the age groups, being approximately 1 per 100,000 men, whereas the incidence of seminoma rose with age. Structurally the most homogeneous tumours were the seminomata, although a small number of cases showed anaplasia and were difficult to differentiate from embryonal carcinoma. The authors believe that seminoma does not arise from spermatogonia but from primordial germ cells; they suggest that this tumour should be called "germinoma." It is emphasized that seminomata differ both structurally and biologically from embryonal carcinomata. The common varieties of the latter type of tumour are those which show glandular and papillary structures (adenocarcinoma and papillary adenocarcinoma), and those which resemble trophoblastic elements, usually cytotrophoblastic and occasionally syncytiotrophoblastic components of chorionic carcinomata.

Chorionic carcinoma (chorion-epithelioma) is regarded as a variety of embryonal carcinoma rather than a separate entity, because chorionic structures were observed also in predominantly embryonal carcinomata and because gynaecomastia and related endocrine changes were associated as often with embryonal carcinomata (or teratocarcinomata) as with chorionic carcinomata. Under the term teratocarcinoma the authors include all tumours which contain differentiated teratoid structures and foci of histologically malignant growth. Most of such tumours examined were mixtures of teratoma, embryonal carcinoma, and or chorionic carcinoma. Teratocarcinoma is thought to result from the teratoid differentiation of embryonal carcinoma. Teratomata without histologically malignant elements should not be termed benign, because they often produce metastases. The metastases of seminomata consisted usually of the same type of cell as the primary growth, but occasionally the metastases showed the characteristics of embryonal carcinoma or teratocarcinoma.

The metastases of embryonal carcinomata were likewise similar to the primary growth, but in some cases the metastases had the structure of chorionic carcinoma, although there was no evidence of this in the primary growth. With teratocarcinomata 40% of the metastases consisted of embryonal or chorionic carcinoma and 60% of teratoid structures. The immediate prognosis is bad in embryonal and chorionic carcinomata and poor in teratocarcinomata and adult teratomata. In seminomata, in comparison with the other tumours of the testis, the immediate prognosis is good. *G. Popják.*

Microscopic Renal Calculi. ANDERSON, L. (1946). *Proc. Mayo Clin.*, 21, 326.

Following up the work of Randall, who in 1941 described the finding of small macroscopic calcareous deposits on the surface of the renal pyramid in 20% of 1,100 pairs of kidneys, the author investigated microscopic calcareous deposits in the substance of the renal papilla. The diameter of these deposits is at least five or six times that of a normal tubular cell. He found them in all of 170 kidneys examined. Some of these organs were apparently healthy and some were diseased; all were removed from bodies more than 9 years of age at the time of death. Discussion of these findings concerns the possibility that these microscopic deposits may form the nidus for the formation of Randall's plaques, and so perhaps for renal calculi in certain circumstances. *Geoffrey Evans.*

The Cytology of Conjunctival Exudates. THYGESON, P. (1946). *Amer. J. Ophthal.*, 29, 1499.

A study of the cytology of conjunctival scrapings, exudates, and follicular expressions may, in combination with the bacteriological report, be of great assistance in making a correct diagnosis in cases of conjunctival disease. The staining method recommended is by Giemsa, 1 drop of stock solution in 1 ml. of distilled water, applied for 1 hour after fixing in absolute methyl alcohol. Excess stain is removed by washing in two changes of 95% alcohol. A polymorphonuclear leucocyte reaction is found in most cases of acute and chronic conjunctivitis, the outstanding exception being diplobacillary infection. An eosinophil reaction is characteristic of all allergic inflammation and is found in hayfever conjunctivitis and in various drug cosmetic allergies as well as in spring catarrh. Mild eosinophilia is found in ocular pemphigus but not in phlyctenular conjunctivitis. There is also a basophil reaction in allergic inflammations of the conjunctiva, parallel with the eosinophilia, but this is not specific because it may also occur in such nonallergic inflammations as trachoma. Mononuclear cells, chiefly lymphocytes, are found in exudates from epidemic kerato-conjunctivitis and acute follicular conjunctivitis. There is a similar reaction to the typical virus infection, such as that due to herpes or molluscum contagiosum. Plasma cells are found in the exudates of trachoma. Keratinization of epithelial cells is found in vitamin A deficiency and also in kerato-conjunctivitis sicca. The author is unable to confirm previous observations that trachoma can be diagnosed from conjunctival cytology

at an early stage, but states that the cytology of expressed trachoma follicles is characteristic. The predominant cell is the lymphoblast, which shows degenerative changes, and there are many macrophages (Leber cells). This typical appearance is due to the necrotizing action of trachoma toxins. *A. G. Cross.*

Anatomical and Histological Lesions of the Liver in Experimental Thyroxine Poisoning and in Hyperthyroidism. (Les lésions anatomiques et histologiques du foie dans l'intoxication thyroxinienne expérimentale et dans l'hyperthyroidisme.) GUYE, P., and RUTISHAUSER, E. (1947). *Ann. Anat. path. méd-chir.*, 17, 1.

This well-documented paper records experiments made on rabbits and post-mortem observations on 28 cases of human thyrotoxicosis. Histologically there is a basic similarity. In the severe cases there is generalized atrophy of the hepatic parenchyma with gross cytoplasmic deprivation (glycogenolysis); in the more extreme cases there is also necrosis, generally central and most obvious in the subcapsular zone. Intoxication of low intensity and short duration produces lesser parenchymatous lesions of reversible degree. If of longer duration, or repeated, the intoxication is followed by fibrosis, both primary (portal in situation) and secondary (in relation to the necrosis). *A. C. Lendrum.*

Histopathology of Polioencephalitis Hemorrhagica Superior (Wernicke's Disease). BAILEY, F. W. (1946). *Arch. Neurol. Psychiat., Chicago*, 56, 609.

Five fatal cases of Wernicke's encephalopathy were studied histologically. All the subjects were said to have been chronic alcoholics, though there is no reference to the amount of alcohol, its nature (except in one case), or the duration of alcoholism. Naked-eye examination of the brain showed cortical atrophy in 4 of the cases and small haemorrhage in the brain-stem in 2. Microscopical examination revealed histological changes confined largely though not entirely to the periventricular grey matter of the midbrain and pons—throughout the hypothalamus, mammillary bodies, medial aspect of the thalamus, inferior colliculi, and to some extent in the floor of the fourth ventricle. In the affected areas the capillaries showed proliferation of endothelial cells and the perivascular spaces were enlarged. There were areas of old and fresh haemorrhages, perivascular and of different sizes. Focal areas of parenchymal necrosis varied from one to several millimetres in diameter. Within the areas of haemorrhage and necrosis, focal neuronal degeneration was present in all cases. In the affected areas astrocytes showed proliferation and degeneration, and to some extent transformation. Oligodendroglia was swollen, with multiplication of cells. It is considered that underlying the histological changes there is a deficiency of nutritional factors, the chief of which is thiamine. It is considered that thiamine lack interferes with carbohydrate metabolism, with resulting anoxia of the tissues.

[This is an interesting study. Although the biochemical explanation may be correct, no suggestions are offered as to why these changes have such a strictly circumscribed distribution.] *Hugh Garland.*

IRON METABOLISM

BY

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(RECEIVED FOR PUBLICATION, JANUARY 5, 1948)

Ten years ago physiologists still taught that the iron metabolism of the body was regulated by a balance between absorption from the stomach and small intestine and excretion by the colon. McCance and Widdowson (1937a) first challenged this theory, arguing from facts already known that there was no evidence of excretion of iron by the body except in very small amounts, and that therefore the iron balance must be maintained by a regulation of iron absorption. In confirmation of this view McCance and Widdowson (1938) showed that when normal subjects were given iron by mouth they excreted an equal amount in the faeces, whereas when iron was given by intravenous injection the whole was retained and none excreted. The logic of this hypothesis compelled its immediate acceptance; but it could not be finally proved until a dose of iron could be "tagged" by the radio-active element so that its whole course through the body could be followed.

The principles of experiments with radio-active elements are now familiar. A minute proportion of the atoms is made radio-active by artificial means. The whereabouts and number of such atoms can be traced by measuring their radio-activity, so that they can be used to "tag" a much greater number of normal atoms, from which they do not differ either chemically or biologically. Radio-active iron is unfortunately one of the more difficult elements to work with, for its radiation is very soft and is readily absorbed by other metals. The radio-active iron must therefore be isolated in a very pure state before it can be measured, and even the metallic iron must be in a very thin layer to avoid errors from self-absorption of radiation.

Physiological Experiments with Radio-active Iron

Radio-active iron appears in the plasma soon after it is fed to a dog and reaches its maximum concentration in four to eight hours. It is, however, quickly taken up by the storage depots, and after a few hours none can be detected in the plasma (Hahn and others, 1939). The iron in the storage depots is very mobile, for radio-active iron can be detected in the red cells within four hours of its administration by mouth. The amount of radio-active iron in the circulating red cells thereafter increases progressively to a maximum which is reached in four to seven days in anaemic dogs (Hahn and others, 1940). The level subsequently remains constant. It can therefore be presumed that all the radio-active iron absorbed has been used in haemoglobin formation, and that none remains in the storage depots.

Much work with radio-active iron has been based on this assumption that iron utilization is a measure of absorption. The supposition is certainly justified when there is iron-deficiency anaemia, in that Dubach and others (1946) have shown that even injected radio-active iron is completely utilized in six to nine days by dogs and men in this condition. In normal animals, however, there is a greater probability that some radio-active iron absorbed from the intestine may be diverted to storage depots and not appear in the circulating red cells during the course of the experiment, but it is unlikely that errors from this source have been great enough to invalidate the major conclusions from the experiments to be described below.

Iron Absorption

Iron is absorbed only in the stomach and small intestine, and not at all in the colon. When a dog is given radio-active iron by mouth the highest plasma level is reached in four to eight hours, and since this is the time when the meal is in the small intestine it is here that the maximum absorption takes place. After eighteen to twenty-four hours, when the whole meal has passed into the colon, no further absorption of radio-active iron occurs (Hahn and others, 1939a). It has also been shown that radio-active iron is absorbed into the blood stream from gastric, duodenal, and jejunal pouches in the dog (Hahn and others, 1943).

A man's daily loss of iron from the body is of the order of only 1 mg., so there must evidently

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be some selective control of iron absorption—otherwise haemochromatosis would be a common complication of old age. The gap between an adequate supply and a deficiency of iron is, however, very narrow, and many factors may inhibit the absorption of iron which the body needs to maintain the haemoglobin level.

The control of iron absorption.—The body absorbs iron according to its needs, in health only enough to replace the small daily loss, but in iron deficiency as much as is offered and physiologically available. It is a common experience that a patient being treated for iron-deficiency anaemia may regenerate haemoglobin at a rate which demands the absorption of 50 mg. of iron a day, whereas McCance and Widdowson (1938) showed that when extra iron is given to a normal individual he excretes it all in his faeces. Hahn and others (1940) showed very clearly the difference in radio-active iron absorption by normal and anaemic dogs. Whereas a normal dog would absorb only very little of a test-dose given by mouth, and a plethoric dog would absorb practically none, dogs suffering from chronic iron-deficiency anaemia might utilize more than half of a small test-dose for haemoglobin production.

However, the degree of iron absorption is not determined by the haemoglobin level *per se*. Thus the intestinal iron absorption of a normal dog is not immediately increased if it is made suddenly anaemic by bleeding, but after a week, when the iron reserves are depleted by haematopoiesis, a test-dose of radio-active iron is vigorously absorbed. Nor will prolonged anoxia increase iron absorption.

Studies in man have given very similar results (Balfour and others, 1942). Normal individuals absorb very little of a test-dose of radio-active iron given by mouth, but absorption is ten times as great in patients with chronic iron-deficiency anaemia. In polycythaemia, on the other hand, only a trace of radio-active iron is absorbed, in spite of the excessive formation of red cells and haemoglobin.

Pregnant women, whether they are anaemic or not, absorb between two and ten times as much of a test-dose of radio-active iron as do normal individuals (Balfour and others, 1942). Here the degree of iron absorption evidently depends upon the demands of the foetus rather than upon any state of anaemia in the mother.

The iron-absorbing powers of the intestine are readily exhausted. Thus a dose of an iron salt given by mouth will diminish the absorption of radio-active iron administered one or two hours

later. The inhibition is not due to the elevation of the plasma iron level by the first dose, for an intravenous injection of iron does not reduce the absorption of radio-active iron given by mouth (Hahn and others, 1943).

Hahn has explained the above facts by postulating that the cells of the intestinal mucosa contain a protein receptor which can receive iron ions from the intestinal contents and yield them to the plasma. Thus the absorption of iron from the intestine depends on the presence of free places in the receptor, and the rate of removal of iron from the intestinal cells into the blood is determined by the level of the plasma iron and its equilibrium with the tissue reserves. The reverse process, a transfer of iron from the plasma to the intestinal cells, is not possible because the plasma iron is attached to a plasma protein, probably globulin, and cannot be yielded to the intestinal receptor. The intestinal mucosa is very readily saturated with iron—within two hours if enough iron is available in the food—but desaturation into the plasma is not complete for some days

Granick (1946) has recently brought forward evidence that the iron receptor in the intestinal mucosa is apoferritin, the protein which binds the greater part of the tissue-storage iron (*vide infra*). Iron combines with apoferritin to form ferritin, and the amount of this compound in the intestinal mucosa increases after feeding with iron, and falls again after several days. There is evidently some local mechanism for the formation or accumulation of apoferritin in the intestinal mucosa, for the total amount of ferritin + apoferritin increases in response to iron feeding.

Very little radio-active iron appears in the red cells of patients with pernicious anaemia when they are given a " tagged " iron salt by mouth. This finding has been interpreted as meaning that the patients, in spite of their severe anaemia, absorb no more iron than do normal people because their iron stores are not depleted. Dubach and others (1946), however, have cogently argued another interpretation. They point out that the blood-level of radio-active iron after a week or so measures only the iron utilized in haemoglobin synthesis, and that much more iron might have been absorbed and stored unused. They have in fact shown this to be the case in a man who had pernicious anaemia without any iron deficiency. In this patient only about 1 per cent of a test-dose of 57 mg. of radio-active iron appeared in the circulating haemoglobin until liver treatment was started nine days later. Then, as the haemoglobin level rose the proportion of the test-dose of radio-active iron in the blood likewise increased

until more than 20 per cent of the administered dose was found in the circulating haemoglobin. Thus the initial low level of circulating radio-active iron was a sign not of small absorption but of deficient haemoglobin synthesis and iron utilization. Dubach and others (1946) have made similar criticisms of the common statement that iron absorption is not increased in haemolytic anaemias.

The theory that iron metabolism is regulated by selective absorption from the stomach and intestine and not by excretion will doubtless demand many similar modifications in detail, but its basic truth still rests on the experiments and reasoning of McCance and Widdowson (1937a, b, 1938, 1943).

Iron valency and absorption.—It is the universal experience of clinicians that in the treatment of iron-deficiency anaemia ferrous salts are effective in about half the dose of ferric salts. Table I (from Witts, 1936) shows the iron content of the average daily doses of ferrous and ferric

TABLE I

IRON CONTENT AND UTILIZATION OF THE AVERAGE EFFECTIVE DOSE OF VARIOUS THERAPEUTIC PREPARATIONS (WITTS, 1936)

Preparation	Iron content of average dose	Per cent utilization
Ferrous chloride ..	100–200 mg.	12.5–25
Ferrous sulphate ..	180 mg.	14
Ferrous carbonate (Blaud's pill)	300–400 mg.	6–8
Ferric chloride ..	400 mg.	6
Ferric citrate ..	400 mg.	6
Iron and ammonium citrate	800–1,600 mg.	1.5–3

salts necessary to raise the total blood iron by 24 mg. per day, equivalent to a haemoglobin increase of about 0.14 g. per 100 ml. It is evident that ferrous salts are absorbed and utilized at least twice as efficiently as ferric salts.

Moore and others (1939) showed that serum iron absorption curves after the administration of iron salts were higher with ferrous than with ferric salts. The difference has been more accurately demonstrated by giving the same subjects successive doses of radio-active ferrous and ferric salts. Table II summarizes some of the experiments of Moore and others (1944). Normal men utilized 1.5 to 10 times as much ferrous as ferric salt, and in hypochromic anaemia the ratio was 2 to 15. Likewise Hahn and others (1945) showed that

patients with hypochromic anaemia utilized 2 to 6 times as much ferrous as ferric salt when given successive test-doses of radio-active iron. Iron utilization can be taken as a measure of iron absorption in iron-deficient subjects if not in normal individuals, so that the superior absorption of ferrous ions is clearly demonstrated.

TABLE II

UTILIZATION OF FERROUS AND FERRIC RADIO-ACTIVE IRON SALTS BY NORMAL MEN AND IN HYPOCHROMIC ANAEMIA (MOORE AND OTHERS, 1944)

Form of iron	Dose given	Normal men		Hypochromic anaemia	
		No. of cases	Per cent utilization	No. of cases	Per cent utilization
Ferrous	4 mg.	4	7	2	12.5
Ferric	4 mg.	4	2.5	2	4
Ferrous	2 mg.	3	7.5	2	27.5
Ferric	2 mg.	3	2	2	4
Ferrous	1 mg.	2	9	1	39.5
Ferric	1 mg.	2	1.5	1	17

The same rule does not apply to all animals. Thus rats absorb ferric salts as well as ferrous (Austoni and Greenberg, 1940), as do many dogs, although the majority of both normal dogs (Moore and others, 1944) and anaemic dogs (Hahn and others, 1945) absorb a greater proportion of bivalent radio-active iron salts.

Moore and others (1944) offered three explanations for the superior absorption of ferrous salts. It is possible that only ferrous salts are capable of absorption, or that ferrous salts are much more readily absorbed than ferric, or that the ferric ions more readily form insoluble compounds in the intestine.

Relation of dosage to iron absorption.—Clinical experience has shown that the response of iron deficiency anaemia to medication depends upon the dose of iron given, and that there is often a minimum " threshold " dose below which treatment is ineffective. It is not clear to what extent this varying response depends upon the proportion of iron absorbed from the intestine, and to what extent upon iron utilization for haemoglobin synthesis. Most experiments with radio-active iron have shown that the higher the test-dose the less is the proportion which appears in the red cells, even though the total utilization may increase (see, for example, Table II). This has commonly been taken to mean that the proportion of iron

absorbed deereases with increasing dose, but it may be rather that with a larger dose a less amount of the iron absorbed is utilized. Brock and Hunter (1937), for example, showed by balance experiments that when large doses of iron were administered by mouth, twice as much iron might be retained in the body as was utilized for haemoglobin synthesis.

Dietary sources of iron.—It is easy to estimate the total iron content of a foodstuff or diet, but it is not usually possible to say what proportion of the iron is potentially available for haemoglobin synthesis. It is now known that only ionic iron can be absorbed from the intestine, but there is no rule for determining how much of the food iron ean be freed in the ionie state by digestion. Iron also very readily forms insoluble eompounds with various constituents of the diet, especially in a medium which is not strongly acid.

It has been amply demonstrated by both clinical and experimental studies that ionic iron is readily absorbed by the stomach and small intestine, but there is no evidence that any pre-formed iron compound can be absorbed as such and used for haemoglobin synthesis. The dietary iron is in two forms—inorganic, which readily passes into solution, and organic, chiefly porphyrin compounds which must be broken down before ionic iron is liberated. The relative proportions of the two can be distinguished by the α–dipyridyl reaction for ionizable iron, and at one time many estimates were made of the " availability " of iron in food by this reaction. Such estimates, however, bear only a very rough relation to the truly physiological availability of dietary iron. Both ferrous and ferric chloride, for example, are " 100 per cent available " according to the α–dipyridyl reaction but neither is completely absorbed from the intestine even in severe iron-deficiency anaemia, and at least twice as much of the ferrous as of the ferric salt is assimilated.

Some of the " non-available " food iron which is not estimated by the α–dipyridyl reaction may also be broken down and absorbed. Thus Blaek and Powell (1942) found that 10 to 20 per cent of haemoglobin iron was absorbed when a litre of eitrated blood was given by duodenal tube. They considered baeterial deeomposition in the intestine to be important in making this iron available.

The chemical nature of iron compounds in foodstuffs is, however, probably less important for their absorption than other even less easily defined factors. Conditions in the small intestine, except in its upper part, are very unfavourable to the absorption of iron. Iron salts are searcely dissociated in solution at a pH above 5.0, so that few ferrous, and even fewer ferric, ions are available for absorption except in distinctly acid medium. It is not surprising, therefore, that iron-deficiency anaemia is commonly associated with gastric achlorhydria.

Iron phosphates are practically insoluble, and the ratio of phosphorus to iron in ordinary diets often exceeds 50, so that there is abundant opportunity for the formation of iron phosphates in the intestine. Phosphates may inhibit iron absorption even when no precipitate is formed, for Brock and Taylor (1934) found that the dialysis of iron salts across cellophane membranes was greatly decreased in the presence of neutral or alkaline phosphates.

Iron forms undissociated compounds with a number of amino-acids and phosphorus-containing substances such as nucleic acid, and McCance and others (1943) have emphasized that an excess of phytic acid in the diet may lead to the deviation of much iron as insoluble phytate.

An excess of ealcium in the diets of animals usually inhibits iron absorption so that a mild iron-deficieney anaemia develops, but under some circumstances an excess of calcium favours iron absorption. Thus the excess phytic acid of wholemeal bread may be neutralized by calcium (McCance and others, 1943), and when the phosphate content of the diet is high a high level of calcium diminishes the formation of insoluble iron phosphates.

The vitamin content of a diet may have a direct influence on iron absorption. Ascorbie acid reduces ferric compounds to ferrous *in vivo* as well as *in vitro*, and thus facilitates absorption (Moore and others, 1939). Extra vitamin D increases haemoglobin formation and iron storage in young rats (Fuhr and Steenboek, 1943).

It is evidently difficult to decide whether a given diet will supply enough iron to maintain the haemoglobin level, and in practice the question is deeided by experience rather than by any scientific knowledge of the physiological availability of iron in various foods. An ordinary mixed diet of the type eaten in England will provide enough iron for a normal man if its total iron content exeeeds 5 mg. daily, and a total of 15 mg. daily will suffice a normal woman or growing child. The average English diet to-day probably gives 15–20 mg. of iron daily, an amount which in theory is rather more than adequate for women and children, and generous for men. The margin for women is small, however, and it requires relatively little disturbance of iron absorption from such causes as

gastric achlorhydria, or relatively little increased iron loss from menorrhagia or frequent child-bearing, to produce an iron deficiency.

The value of foodstuffs as iron sources is similarly a matter of empirical rather than scientific knowledge. Liver, eggs, dried fruits, chocolate, sardines, and green vegetables are all good sources, but nowadays are not freely available. Oatmeal, peas, beans, and whole-meal bread are also good sources, but white bread contains less than one-third of the original iron of the grain. Such staple foods as meat, fish, chicken, milk products, and beer are relatively poor iron sources.

Iron Transport

The plasma is the chief medium for the transport of iron, which is carried firmly bound to a plasma protein, probably globulin. It is, however, uncertain whether iron for haemoglobin synthesis is thus carried to the bone marrow as ferric ions, or as some pre-formed compound.

Iron is absorbed chiefly, and perhaps entirely, as ferrous ions, but the oxygen tension of the plasma is sufficient for the immediate conversion of the ions to the ferric state. The plasma iron is probably bound to the serum globulin as a ferric hydroxide complex.

Powell (1944) found the mean plasma iron of normal men to be 143 γ (S$=24$ γ), whereas the level in women was significantly lower, with a mean of 117 γ (S$=27$ γ). There was a significant reduction in the plasma iron of women during menstruation.

The plasma iron rises sharply during the absorption of a dose of iron from the intestine. The height of the increase depends upon the rate of deposition of iron in the tissues as well as upon the rate and amount of intestinal absorption, but plasma iron curves can be used to demonstrate whether iron is in fact being absorbed, and to obtain a rough idea of the relative absorption of different compounds. Moore and others (1939) have shown that the rise in the iron content of the plasma during absorption is not preceded by an increase in the iron content of the thoracic duct lymph, so that iron must pass directly from the intestinal mucosa into the blood stream.

The plasma iron is reduced below normal in patients with iron deficiency, and also during periods of active erythropoiesis such as occur after acute haemorrhage or during the cure of pernicious anaemia. Conversely, the plasma iron is usually high when erythropoiesis is depressed, as in untreated pernicious anaemia or hypoplastic anaemia. The level is very variable in haemolytic anaemias, depending upon the balance between red cell destruction and haemoglobin synthesis.

Easily split-off iron.—About 5 per cent of the blood iron can be easily dissolved by digestion with decinormal hydrochloric acid, and it was believed until recently that this "easily split-off iron" might be of importance in iron transport. Sixty to seventy per cent of the easily detached iron is derived from the red blood cells, but if the haemoglobin is converted into carboxyhaemoglobin this iron can no longer be removed by acid digestion. Legge and Lemberg (1941) have shown that this fraction is an artefact—when red blood cells are digested with acid, oxygen is liberated from its combination with haemoglobin and oxidizes a variable amount of the corpuscular haemoglobin into open-ring compounds similar to the iron-containing bile pigments, from which iron is easily removed by acid digestion. The remainder of the easily detached iron is derived from bile pigment-haemoglobin, blood catalase, and the plasma iron.

Iron Storage

It is evident from clinical observation that the body normally holds a considerable reserve of iron readily available for haemoglobin synthesis. A patient who, through acute haemorrhage, has lost a third or more of his circulating red cells may rapidly regain a normal haemoglobin level without any assistance from iron therapy, although the loss of 700 mg. or more of iron could scarcely be replaced from the diet within six months. We have little knowledge of the size of this available iron reserve. The greater part of the body iron is normally circulating as haemoglobin, and perhaps half of the balance is in the form of myohaemoglobin and cell-enzymes such as cytochrome, and is not available for haemoglobin synthesis. Table III shows Hahn's (1937) estimate of the iron

TABLE III

PERCENTAGE DISTRIBUTION OF THE TOTAL BODY IRON OF A NORMAL DOG (FROM THE DATA OF HAHN, 1937)

Circulating iron	Available reserves	Non-available iron
Blood haemoglobin.....57%	Liver, spleen, and marrow .....15% Elsewhere ...5%	Myohaemoglobin 7% Parenchyma iron......16% (cell enzymes, etc.)
Total 57%	20%	23%

distribution in a normal dog. On a similar basis a normal man would have an available iron reserve of about 850 mg., sufficient to replace about one-third of his circulating haemoglobin.

The greater part of the iron reserve is in the liver, spleen, and bone marrow. Much of the iron in the marrow is present as haemoglobin in formed or immature red blood cells, whereas the reserve iron in other tissues is probably held in the reticulo-endothelial cells.

Many attempts have been made to gain further knowledge of the iron reserves by injecting colloidal iron intravenously into animals, but it is at least possible that the animal treats iron in this form as a foreign particle rather than as a normal dissolved metabolite.

The chemical form of the iron reserves is still not completely understood. Haemosiderin, which histologically is so prominent a product of red-cell destruction, is certainly not the major iron reservoir. It is probably an intermediate stage or side product in the conversion of haemoglobin into bile pigment, but little is known of the chemical reactions involved.

Ferritin.—Iron-storing tissues contain a protein, apoferritin, capable of combining with as much as 23 per cent of its weight of iron. The iron is firmly held to the surface of the apoferritin molecule as small micelles of ferric hydroxide–ferric phosphate to make a protein, ferritin, of variable iron content. Both ferritin and apoferritin can easily be crystallized as the cadmium salt, and minute quantities of either can be detected by a precipitin reaction with an anti-apoferritin serum.

Studies with radio-active iron have shown that at least a part of iron which is injected is quickly converted into ferritin, and that when "tagged" red cells are lysed the liberated iron can be detected in ferritin. It seems probable that ferritin functions as the most important, though not the only, store of iron readily available for haemoglobin synthesis. It may also be the intestinal iron receptor (*vide supra*). The chemical properties and functions of ferritin have been well reviewed by Granick (1946).

Some of the earliest studies with radio-active iron showed that the iron liberated by destruction of effete red blood cells was immediately re-used for haemoglobin synthesis in preference to the reserve iron stores (Hahn and others, 1942 ; Cruz and others, 1942). Greenberg and Wintrobe (1946) have pointed out that when a small quantity (about 5 mg.) of radio-active iron is injected into a normal individual, about 15 per cent of the dose is built into haemoglobin each day. If the injected

iron merged with the whole available iron reserve the utilization should be only 2 to 3 per cent daily, whereas if it were used in proportion to the daily liberation of iron from effete red cells the daily utilization should be about 83 per cent. It therefore appears that about one-quarter of the iron reserves form a "labile iron pool" used for normal haemoglobin synthesis. Dubach and others (1946) have also shown that injected iron, and presumably, therefore, iron from recently lysed red cells, is used for haemoglobin synthesis in preference to the iron reserves.

Iron Excretion

McCance and Widdowson (1937a, 1938) first demonstrated that iron excretion in man is too small to play any significant part in the control of iron metabolism. They pointed out that the faecal iron output varies with and nearly equals the food iron, and that when iron is injected practically none is excreted. In one dramatic experiment (McCance and Widdowson, 1937b) they treated a polycythaemic patient with acetyl-phenylhydrazine and reduced his haemoglobin level from 168 to 47 per cent Haldane. They calculated that over 6 g. of iron was liberated from the lysed red cells, yet less than 0.5 per cent of this amount was excreted. More recently (1943) the same authors transfused 28 pints of blood into a patient with haemolytic anaemia in a hundred days. All this blood was haemolysed, liberating about 80 mg. of iron a day, yet the total daily iron excretion (5.2 mg.) was slightly less than the dietary intake (5.6 mg. per day), so that none of the iron freed by haemolysis can have been excreted.

Hahn and others (1939b) followed the fate of radio-active iron injected as gluconate into dogs. The urinary excretion was negligible, apart from a "spill-over" immediately after the injection, and the faecal excretion was as small in plethoric as in anaemic dogs. Any considerable haemolysis of red cells increased the faecal iron excretion, and the authors received the impression that most of the iron thus excreted was derived from the bile.

Biliary iron excretion.—Hawkins and Hahn (1944) have recently studied the biliary excretion of iron by establishing biliary fistulae in dogs. After determining the basal daily iron and bile pigment excretion they produced severe haemolysis with acetyl-phenylhydrazine. The biliary iron and pigment excretion rose in parallel, sometimes as much as tenfold, so that it was reasonable to conclude that the biliary iron, like the pigments, was derived from the breakdown of haemoglobin The iron was probably a part of the pigments, for

iron injected intravenously was not excreted in the bile.

Hawkins and Hahn concluded from their experiments that about 3 per cent of the iron freed by haemolysis was excreted in the bile, and that the remainder was stored or re-used for haemoglobin synthesis.

Urinary iron excretion.—The importance of the urinary iron excretion is still uncertain. The careful analyses of Barer and Fowler (1937) suggest that the normal daily excretion of iron in the urine of men is 0.4 mg. and in women 0.5 mg.—a substantial proportion of the total daily loss. Other workers have regarded the urinary iron excretion as negligible, and derived from cell debris rather than from ionic transfer across the glomerular membrane.

Daily Iron Requirements

The minimum daily iron requirements are evidently equal to the total loss from the body by excretion in the bile and urine, desquamation of cells, and haemorrhage. The necessary dietary intake, in order to allow for unabsorbed iron, must of course exceed these physiological requirements, but a calculation of the latter provides a basis for comparing the needs of different classes of individuals.

Normal men.—An average man has 6 litres of blood with a haemoglobin content of 15.5 g. per 100 ml., so that his total circulating haemoglobin is 930 g. If we take the average life of the red cell to be 120 days, the daily breakdown of haemoglobin is $930/120 = 7.75$ g., containing 26 mg. of iron (1 g. of haemoglobin contains 3.34 mg. of iron). If we assume that man, like the dogs of Hawkins and Hahn (1944), excretes 3 per cent of the liberated iron in the bile, the daily loss by this route is 0.8 mg. Urinary iron excretion, according to Barer and Fowler (1937), amounts to 0.4 mg. daily, giving a total iron excretion of 1.2 mg. per day (Table IV).

TABLE IV

DAILY IRON LOSSES OF NORMAL MEN AND WOMEN

	Men (mg.)	Women (mg.)
Biliary excretion	0.8	0.6
Urinary excretion ..	0.4	0.5
Menstruation	—	1.0
Total	1.2	2.1

Normal women.—A similar calculation can be made for normal women, but the iron loss of menstruation must be added. McCance and Widdowson (1937a) found by direct analysis that normal women lost 10–40 mg. of iron in each menstrual period, so that the average loss can be expressed as about 1 mg. per day over the whole menstrual cycle. A woman must therefore replace nearly twice as much iron each day as a man (Table IV), and even moderate menorrhagia may raise the requirements to 5 or 6 mg. daily.

Child-bearing.—Davidson and Fullerton (1938) calculated that a pregnant woman contributes 400 mg. of iron to the foetus and 150 mg. to the placenta and uterus. A blood loss in parturition of about 500 ml. accounts for a further 175 mg. of iron, making a total of 725 mg. The greater part of this iron drain occurs towards the end of pregnancy when the foetus is laying down most of its iron stores, but for convenience of comparison the iron loss may be expressed as an average of 2.7 mg. per day throughout the pregnancy. The total iron demands of a pregnant woman are thus about 3.8 mg. daily—nearly twice as much as a non-pregnant woman needs.

The iron drain of lactation is comparatively light, about 1.5 mg. a day, but when, as often happens, menstruation recommences during lactation the total iron loss is almost as great as in pregnancy.

Children and adolescents.—The increasing blood volume and tissue iron of children makes a considerable addition to their iron needs. Heath and Patek (1937) calculated the annual iron requirements for the increase in blood volume and parenchyma iron in each year of life ; their figures make no allowance for any increase in the available iron stores. The additional iron thus laid down in the first year of life amounts to 200 mg., but the figure falls to 100 mg. during the third year and does not rise again appreciably until the ninth year, when the growth rate begins to increase. The amount of iron laid down annually then begins to rise to a maximum of 350 mg. during the seventeenth year, and then falls to zero as growth stops at about the twenty-first year. Thus the extra iron demands of growth never exceed 1 mg. a day, but even this is a substantial addition to the needs of a menstruating girl.

Clinically it appears that the iron needs of children are greater than these calculations admit, and it may be that their greater metabolic rate increases their iron needs.

Iron Losses from Haemorrhage

The reserve powers of the bone marrow are great enough to cope with haemorrhage at a rate of at least 150 ml. of blood a day, provided enough iron is available. A normal man replaces the red cells of 50 ml. or so of blood every day and only uses about one-quarter of his total marrow capacity, but his daily iron loss is only of the order of 1 ml. a day—the iron content of 2 ml. of blood. Even if the amount of iron absorbed from an ordinary diet were 20 mg. a day, a very improbable figure, the marrow would not receive enough iron to double its output. It is not, therefore, the loss of blood which prolongs the anaemia of haemorrhage, but the loss of iron.

Acute haemorrhage.—The blood loss of acute haemorrhage not exceeding one-third of the blood volume is usually quickly made good with the aid of the body's iron reserves, but it is well to remember how enormous is the iron loss in relation to the potential dietary intake. A pint of blood contains about 90 g. of haemoglobin and 300 mg. of iron, and even if the diet were capable of yielding an extra 5 mg. of iron a day in excess of a woman's normal requirements (2 or 3 mg. daily), it would take two months to replenish the iron reserves.

When repeated acute haemorrhages reduce the haemoglobin to below 50 per cent of its normal value the iron reserves will certainly be exhausted before recovery is complete, and unless extra iron is given the final rate of recovery will be limited by the available iron of the diet to something less than 2 per cent per week.

It is evident that iron therapy may be given with advantage during convalescence from acute haemorrhage, for with adequate dosage the body can absorb and utilize more than 50 mg. of iron a day. The idea is familiar to physicians, who most often deal with acute haemorrhage from peptic ulcer, often complicated by previous iron deficiency from chronic haemorrhage, but surgical and obstetrical patients also would probably benefit from iron therapy.

Chronic haemorrhage.—Relatively slight chronic haemorrhage greatly increases the iron losses of the body. Normal blood contains about 50 mg. of iron per 100 ml., so that the loss of only 10 ml. of blood a day increases the iron demands of a man fivefold. The drain of iron may to some extent be diminished by the development of anaemia, for the haemoglobin level and iron content of the blood fall in parallel.

It is uncertain how much haemoglobin iron is reabsorbed when chronic bleeding occurs into the small intestine. The question is of great importance in tropical countries, where iron deficiency from hookworm infestation is a major cause of ill-health. A load of 100 hookworms is only moderate, and will not always cause anaemia, yet, if we accept the usual estimate, each worm withdraws 0.5 ml. of blood a day, making a total iron loss of 25 mg. daily. With really heavy infestations of 1,000 or more worms anaemia is almost inevitable, but even if the blood haemoglobin level is reduced to 3 g. per 100 ml. the blood withdrawn by the worms will contain 50 mg. of iron each day. It is scarcely possible for the whole of this deficiency to be replenished from the diet, so that unless the worms are less voracious than is commonly believed a great part of the haemoglobin iron must be reabsorbed from the intestine.

If, on the other hand, enough iron is supplied the reserve function of the bone marrow is sufficient to maintain the haemoglobin level in spite of chronic haemorrhage, unless prolonged anaemia has led to marrow hypoplasia.

Iron Therapy in Anaemia

It is possible to estimate the actual utilization of iron during recovery from anaemia if the changes in blood volume as well as in haemoglobin level are known. Some examples of such calculations are given below in order to emphasize how great is the amount of iron used in raising the haemoglobin to normal levels.

Iron-deficiency anaemias.—Table V shows the blood volume and haemoglobin level of a young woman suffering from a simple iron-deficiency anaemia. During the first nine days of treatment the total circulating haemoglobin increased by 90 g., corresponding to 300 mg. of iron, an increase of 50 per cent. The blood count did not change during the first four days of treatment, so from the fifth to the ninth day 60 mg. of iron were

TABLE V

TOTAL HAEMOGLOBIN VALUES IN A WOMAN
WITH IRON-DEFICIENCY ANAEMIA

Day of treatment	0	9	23	49
Haemoglobin g. per 100 ml.	6.7	8.5	10.5	13.3
Plasma volume ml. ..	2,120	2,250	2,410	2,100
Blood volume ml. ..	2,830	3,310	3,700	3,690
Total haemoglobin g.	190	280	390	490

Patient's weight: 50 kilogrammes.

being utilized for haemoglobin synthesis each day. Since the anaemia was due to a complete exhaustion of the patient's iron reserves, all of this iron must have been absorbed from the intestine. Exsiccated ferrous sulphate was being given in a dose of 1.8 g. (27 gr.) daily, containing 600 mg. of iron, 10 per cent of which was being absorbed and utilized. At the end of seven weeks the haemoglobin had still not quite reached a normal level, but the total amount in circulation had increased by 300 g., corresponding to 1,000 mg. of iron. Over the whole period of treatment the patient had utilized iron at an average rate of 20 mg. a day.

Before this patient was treated she had a normal plasma volume and, consequently, a low blood volume, so that her haemoglobin level underestimated her total iron deficiency. Her cure demanded not only a return of the haemoglobin level to normal, but also an increase of the total red cell volume to give a normal blood volume. Thus whereas during the course of treatment her haemoglobin level rose to 100 per cent above its original level, the total circulating haemoglobin increased by 160 per cent. These figures illustrate how great may be the discrepancy between the true iron utilization and the apparent utilization calculated simply from the haemoglobin level without regard to the changing blood volume.

Pernicious anaemia.—Theoretically, iron should not be necessary in the treatment of pernicious anaemia, for the haemoglobin level was once normal, and nearly all the iron from the missing red cells is retained in the body. In practice, however, iron deficiency, as shown by a mean corpuscular haemoglobin concentration below 32 per cent, is often present when the anaemia is first diagnosed, or else develops during treatment. Iron deficiency is so common in women that in them it must often precede pernicious anaemia, but the frequent development of iron deficiency during the treatment of pernicious anaemia in men is not so readily explained. Dubach and others (1946) have shown that recently injected radioactive iron is more readily utilized than the tissue iron reserves during the cure of pernicious anaemia, and it may be that much of the great excess of iron in the tissues is not readily available for haemoglobin synthesis.

Table VI indicates the great amount of iron which may be utilized during the cure of a case of pernicious anaemia. The patient, an old woman, had a total blood volume which was low for her weight, though the plasma volume was definitely above the normal. Her total circulating haemo-

TABLE VI

TOTAL HAEMOGLOBIN VALUES IN A WOMAN
WITH PERNICIOUS ANAEMIA

Day of treatment	0	50
Haemoglobin g. per 100 ml. ..	3.9	14.0
Plasma volume ml.	3,070	2,300
Blood volume ml.	3,470	3,800
Total haemoglobin g.	130	530

Patient's weight: 45 kilogrammes.

globin rose during treatment from 130 to 530 g., an increase utilizing 1.34 g. of iron. The patient was a very small woman, weighing only 45 kilos, and on the same basis a man of 70 kilos with pernicious anaemia of the same severity would utilize about 2 g. of iron in raising his haemoglobin to normal.

These figures emphasize the necessity for watching for the development of iron deficiency during the treatment of pernicious anaemia, or, if frequent haematocrit investigations are not possible, of supplementing the liver therapy with adequate doses of iron.

The Anaemia of Infection

Infections in man are commonly complicated by a normocytic or slightly microcytic anaemia which is very resistant to treatment until the infection is cured. The frequency of hypochromia in this type of anaemia suggests that it is at least in part due to some disturbance of iron metabolism, and Cartwright and others (1946a, b) have recently done much to confirm this view. They found that the plasma iron was markedly diminished during infections, and that the hypoferraemia persisted until the infection was cured, even though there was no anaemia. The fall in the plasma iron might be evident within two or three days of the onset of an acute infection. When 1 g. of ferrous sulphate was given by mouth the plasma iron level did not rise, although in normal subjects such a dose would increase the level twofold. Even an intravenous injection of iron disappeared from the blood with abnormal rapidity, so it seemed that the anaemia was due at least in part to a deviation of iron from the bone marrow to some other site —probably the infected tissues.

Hypoferraemia followed by anaemia was found when staphylococcal or sterile turpentine abscesses were produced in dogs, but neither staphylococcal toxin nor killed typhoid vaccine produced these changes.

Hahn and others (1946) have also shown that anaemic dogs utilize radio-active iron much more

slowly after the induction of a sterile turpentine abseess than before.

We still do not know whether this deviation of iron from blood formation to infected tissues is a feature of the defenee mechanism or simply a toxic reaction.

Summary

There is no evidenee of exeretion of iron by the body except in very small amounts, and the iron balance must therefore be maintained by a regulation of iron absorption. The greater part of the iron freed from broken-down red cells is re-used for haemoglobin synthesis, and only about 1 mg. a day is exereted in the bile and urine. Even the iron losses of normal menstruation, pregnaney, and laetation do not raise the average daily loss above 4 mg.

Iron absorption from the intestine varies, in accordance with the body's needs, from the 1 mg. or so a day necessary to maintain the iron balanee of a normal man, to the 50 mg. or more a day which may be absorbed and utilized during intensive treatment of iron-deficieney anaemia. The amount of iron absorbed is determined by the iron level in the eells of the intestinal mueosa, where a protein reeeptor, probably ferritin, ean receive iron from the intestinal contents and yield it to the plasma. Iron is transported by the plasma to the tissue stores, and the equilibrium between the iron level in the stores, the plasma, and the intestinal receptor determines the saturation of the last and the degree of iron absorption from the food. Anaemia *per se* has no influence on iron absorption.

Iron can be absorbed from the stomaeh and small intestine so long as the reaetion is aeid. Only ions are absorbed, so that the non-ionizable porphyrin iron of the food must be broken down by digestion or bacterial deeomposition before it ean be assimilated. It is possible that only ferrous ions ean be absorbed, and they are certainly much more readily absorbed than ferrie ions, but the latter are also more apt to form insoluble compounds in the intestine. The value of a diet as an iron source is largely determined by the amount of substances, such as phosphates, eertain protein derivatives, and phytic acid, which form undissoeiated compounds with iron. As a general rule, however, an ordinary mixed diet suffiees for a man if it contains a total of 5 mg. of iron, or for a woman or growing child if it contains 15 mg.

Some 60 per cent of the total body iron is circulating as haemoglobin in the blood, and a further 20 per eent is held in the tissues in a form potentially available for haemoglobin synthesis. The greater part of this storage iron is present as ferritin. Iron recently absorbed or liberated from destroyed red cells is used for erythropoiesis in preference to the iron reserves, and about one-quarter of the latter seems to be more immediately available than the rest.

Of the remaining body iron, about one-third is in myohaemoglobin and about two-thirds in such eell enzymes as eytoehrome. None of this iron is available for haemoglobin synthesis even in extreme iron depletion, but beyond this scarcely anything is known of its metabolism.

The iron losses of even moderate haemorrhage are very heavy in relation to the body's usual needs. A man normally absorbs only a milligramme or so of new iron a day, whereas 100 ml. of his blood contains about 50 mg. of iron. A blood loss up to about one-third of the total blood volume ean be replaeed from the iron reserves if these are saturated, but any greater loss must be replaeed from the diet, whieh if unsupplemented will allow a haemoglobin inerease of only some 2 per cent per week. The iron loss of chronic haemorrhage can easily exeeed the eapaeity of an ordinary diet, but if the bleeding is into the stomach or duodenum a part of the haemoglobin iron can be re-absorbed.

Recovery from severe anaemia may utilize 2 g. of iron, a variable amount of whieh can be taken from the body's reserves. At the one extreme, in iron-deficieney anaemia all the iron must be absorbed; at the other, in pernieious anaemia the iron reserves are theoretically adequate to raise the haemoglobin to normal, although in practice the whole reserves are apparently not always readily available.

The anaemia of infeetion is associated with a low plasma iron, and there is evidenee that iron is deviated from blood formation to the site of infection.

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THE ERROR OF COLOUR MATCHING WITH HALDANE'S HAEMOGLOBINOMETER

BY

JOHN L. D'SILVA AND E. C. TURTON

Department of Physiology, St. Bartholomew's Hospital, London

Haldane and Smith (1899–1900), in the description of their well-known technique for the determination of haemoglobin in blood, realized that the comparison of the tints of solutions of carboxyhaemoglobin contained in tubes may be inaccurate. They wrote as follows (p. 334): ". . . when the tints are really equal the tubes may look unequal, the apparent inequality being, however, in the other direction when they are transposed. This commonly affects not merely the depth, but also the quality of the tints. Errors of such a kind are avoided by repeatedly transposing the tubes during the comparison." In the same communication (p. 333), they stated: "The points at which there was just too little, and just too much, water were noted, and the mean taken as the correct result."

This advice, to judge from a recent publication, is often overlooked. Macfarlane (1945, p. 65) inquired of sixty "competent observers" engaged in haemoglobin surveys for the Medical Research Council as to the precise manner in which they used the instrument. Thirty-nine stated that they transposed the standard and unknown tubes as advised by Haldane, whereas seventeen did not transpose the tubes, and four did not reply to the inquiry. It appeared further that, of these sixty observers, only five adopted Haldane's advice in its entirety.

We have investigated the accuracy with which two observers (J.L.D. and E.C.T.) could match the tints of solutions of carboxyhaemoglobin under different experimental conditions. The results of these experiments are in the course of publication elsewhere. They may be stated briefly as follows:

(a) When two identical, ungraduated, haemoglobinometer tubes containing solutions of carboxyhaemoglobin are viewed side by side, and touching against a background of an illuminated ground-glass screen, the observer may judge the solution on the right to be "too dark" (that is, strong) when, in fact, it is the weaker of the two.

(b) The error alters as the distance between the observer's eyes and the comparison tubes alters.

(c) At any one distance the error changes if the intensity of illumination is changed, or if a daylight bulb is substituted for a "clear" bulb as the source of light.

In the present series of experiments, the errors of twenty observers (six pathologists, four senior technicians, and ten medical students) were investigated.

Plan of Research

The following groups of experiments were carried out.

(a) Each observer made a series of comparisons in daylight.

(b) Each of the ten students made two series of comparisons, (i) with the eyes at 40 cm., and (ii) with the eyes at 125 cm. from the comparison tubes, the source of light being a "clear" electric bulb set up as described below.

(c) Conditions were found in which the student observers could compare the tints of solutions of carboxyhaemoglobin with considerable accuracy.

The Experimental Method

A series of nine dilutions of carboxyhaemoglobin was made up from a 2 per cent stock solution of blood whose haemoglobin content was 100 per cent (Haldane). The tint of the solution labelled "1" approximately matched a Haldane standard. The remaining eight solutions differed from one another and from the solution "1" by steps of 2 per cent. The strongest solution was labelled 1.08, and the weakest 0.92.

The same two ungraduated haemoglobinometer tubes of average internal diameter 6.60 and 6.63 mm. were used in all these experiments, and in every comparison of tints the same tube always occupied the left-hand position. The tubes were not transposed. The tubes were filled by an experimenter with any pair of the nine available solutions of carboxyhaemoglobin. The observer's part in the experiment was solely to report whether, in his opinion, the solution in the right-hand

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From the observations, the ratio of the concentrations of the solutions in the right- and left-hand tubes which gave the observer an equal chance of recognizing a difference in tint correctly was calculated. This was called the "50 per cent ratio." Thus, if the 50 per cent ratio is 1, it means that the observer did not bias his observations in favour of either tube. On the other hand if it is 0.95, it means that the observer judged the solution in the right-hand tube to be "strong" when, in fact, it was "weak." The value of the 50 per cent ratio, therefore, is a measure of an observer's error in tint discrimination.

The method of calculation is based on that used for determining the LD50 for a drug (*cf.* Gaddum, 1933), Behrens's (1929) calculation to allow for the smallness of the sample being employed. When the normal equivalent deviation (N.E.D.) of the percentage of correct observations at each ratio tested is plotted against the ratio concerned, the points lie on or near a straight line. The ratio corresponding to zero N.E.D. represents the ratio at which the observer has an equal chance of discriminating between the tints of the two solutions. The standard deviation (S.D.) of the 50 per cent ratio was calculated from formula 13 on p. 27 of Gaddum's (1933) paper. The application of the method is described in detail in another communication (D'Silva and Turton).

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The mean distance (black rectangle), the least, and the greatest distance at which observers made their comparisons in daylight. The observers' numbers correspond to those in Table I. The number of observations is given at the head of each group of three rectangles.

3.5 ft. from the bulb (illumination of 10 f.c. approximately) and the comparisons of tint were made against it. The room was darkened apart from the light referred to above. The comparisons were made as described previously.

Each of the student observers carried out a set of comparisons with the eyes at (*a*) 40 cm. and (*b*) 125 cm. from the tubes. The results are in Table II.

In every subject, except P.J.L., R.Z., and W.R.C.B., the 50 per cent ratio changed when the distance between the eyes and the comparison tubes was altered from 40

to 125 cm. The change in distance can affect the error of comparison in three ways: (*a*) the 50 per cent ratio may be unchanged; (*b*) it may become less; or (*c*) it may become greater. Of the observations made at 40 cm., only three observers (R.H.G.B., C.C.M., and P.H.S.) had 50 per cent ratios not significantly different from 1. All the observers (except R.Z. at 40 cm.; *cf*. Table II) were consistent in their observations, though only three were accurate. Their results showed that the precise difference in concentration between the solutions in the two tubes which led to difficulty in distinguishing between the tints was different for different observers.

Conditions under which Accurate Comparisons were made by some Observers

In this series of experiments (results in Table III) we tried to find conditions under which the observers' 50 per cent ratios would not differ significantly from 1. Three of them (R.H.G.B., C.C.M., and P.H.S.) made accurate tint comparisons at 10 f.c with the eyes 40 cm. from the tubes (*cf*. Table II). The results of the other seven observers show that when the distance of comparison was altered from 40 to 125 cm., in three (D.M., A.J.W., and G.C.H.C.) the 50 per cent ratio increased, in one (D.E.W.) it diminished, and in three (P.J.L., R.Z., and W.R.C.B.) it was unchanged.

Of the three observers for whom the 50 per cent ratio increased, in two it changed from less than 1 to more than 1. For these two (D.M. and A.J.W.) we found empirically that accurate tint discrimination

TABLE II

ERRORS OF COMPARISON OF TEN STUDENT OBSERVERS WITH AN ILLUMINATION OF ABOUT 10 F.C. PROVIDED BY A 60-WATT "CLEAR" BULB

Observer	50% ratio and 2 S.D.	
	40 cm.	125 c.m.
R.H.G.B.	0.999 ± 0.012	1.044 ± 0.016
C.C.M.	1.005 ± 0.012	1.056 ± 0.017
P.H.S.	1.006 ± 0.015	0.950 ± 0.010
P.J.L.	0.980 ± 0.011	0.977 ± 0.013
D.M.	0.968 ± 0.009	1.027 ± 0.022
A.J.W.	0.967 ± 0.015	1.057 ± 0.014
D.E.W.	0.955 ± 0.012	0.912 ± 0.015
R.Z.	0.954 ± 0.040	0.940 ± 0.017
W.R.C.B.	0.945 ± 0.014	0.958 ± 0.013
G.C.H.C.	0.924 ± 0.022	0.960 ± 0.014

TABLE III

CONDITIONS OF ILLUMINATION AND DISTANCE WHICH ALLOWED SEVEN UNTRAINED OBSERVERS TO COMPARE THE TINTS ACCURATELY: C = "CLEAR" BULB; D = "DAYLIGHT" BULB

Observer	Illumination in f.c.	Dist. in cm. of eyes from tubes	50% ratio and 2S.D.
R.H.G.B.	C 10	40	0.999 ± 0.012
C.C.M.	C 10	40	1.005 ± 0.012
P.H.S.	C 10	40	1.006 ± 0.015
D.M.	C 10	50	0.991 ± 0.009
A.J.W.	C 10	60	0.995 ± 0.014
D.E.W.	C 100	40	0.991 ± 0.009
G.C.H.C.	D 10	85	1.007 ± 0.014

was achieved when the distance of comparison was 50 cm. and 60 cm., respectively. On the same basis, G.C.H.C. presumably needs a longer comparison distance than 125 cm. (*cf.* Table II), but comparisons made at distances greater than 125 cm. are less accurate. When illumination was provided by a daylight lamp, however, G.C.H.C. was accurate at a comparison distance of 85 cm. (Table III).

In the case of D.E.W., the 50 per cent ratio diminished as the comparison distance increased and was always less than 1 at 10 f.c. We found no distance at which this observer could make accurate comparisons at 10 f.c. At 100 f.c., however, he made accurate comparisons when his eyes were 40 cm. from the tubes.

The remaining three observers (P.J.L., R.Z., and W.R.C.B.), for whom a change in comparison distance was without significant effect on the 50 per cent ratio, were not dealt with further. P.J.L. had only a small error (2 per cent ± 1 per cent) in his 50 per cent ratio at 40 cm. and it was felt that any significant change in this in the direction of greater accuracy would be difficult to establish. W.R.C.B. had a large error, but we could not find any conditions under which his 50 per cent ratio was near 1. It was later discovered that he had uncorrected astigmatism which may have contributed towards his inaccuracy. R.Z. at 40 cm. (Table II) had a wide scatter in his observations (as is shown by a high S.D.), and though he was more consistent at 125 cm. he was just as inaccurate.

Commentary

Three points arise from this work in connexion with the determination of haemoglobin.

(*a*) All but one of our trained observers made appreciable errors in comparing the tints of solutions of carboxyhaemoglobin. They were no more accurate than our untrained observers and, as we have shown previously, the inaccuracy is not due to instrumental errors. It seems probable that it is the result of the make-up of the individual's visual apparatus. None of our observers was aware, during the course of the experiment, of any inaccuracy of comparison, so for accurate work it would be advisable for workers who do not transpose the haemoglobinometer tubes during their tint-matching procedure to undertake experiments to determine the magnitude of their errors.

(*b*) Macfarlane's recent communication (1945, p. 67) shows that six different methods of haemoglobinometry were in use by 56 observers, namely: (i) standard always kept on the left, (ii) standard always kept on the right, and (iii) standard first on one side and then on the other. All the observers using these three methods took the first observation of "match" to be the true match point. The observers (iv), (v), and (vi) used the methods (i), (ii), and (iii), respectively, in their tint-matching procedure, but took the mean of the last observation of "strong" and of the first observation of "weak" as the true match point.

We can illustrate the possible errors which can be made by an observer by considering one with a 50 per cent ratio of 0.90, that is, one who judges the right-hand tube to be 10 per cent stronger than it is. Let us assume that he uses the experimental procedures (i) to (vi) (above) in turn, and further that the true match should be at 100 on the Haldane scale.

If he keeps the standard always on the left, he will match at 110. If he keeps the standard always on the right, he will match at 90 for the same reason. If he uses (iv) or (v) his match points are more likely to be 112 and 92, respectively, because the difference between his last observation of "strong" and his first observation of "weak" will be 4 per cent or 6 per cent on the Haldane scale. In each of these four experiments he will have a large error solely because of his inaccuracy in tint matching.

Now consider the situation when the same observer examines the unknown solution first on the right of and then on the left of the standard. Assume that in the course of the experiment, dilution has reached the "90-mark." When the standard is to the left of the unknown, the latter will be recorded as strong. When the positions of the tubes are reversed, however, a match may be recorded because of the observer's 10 per cent error. The observer will be faced with a dilemma in which the same solution appears to be strong when it is viewed on one side of the standard, and a match when it is viewed on the other side. Two courses only are open to him. Either (*a*) he can record his judgment as a non-committal "match" and continue his observations, finally taking the mean of the readings at which he judged the unknown to be just too strong and just too weak as the true match point (*cf.* procedure (vi), above); or (*b*) he can judge the dilution at which the unknown appears as strong, when it is viewed on one side of the standard, as it appears weak when it is viewed on the other, and call this the true match point. The latter procedure introduces uninvestigated factors concerning judgment and memory for differences of tint and so, at present, must be regarded as unreliable. It is likely, therefore, that the observer will obtain the most accurate results when he examines the unknown first on the right of and then on the left of the standard and takes the mean of the last

observation when the unknown appeared strong whether it was viewed on the right or the left of the standard, and the first observation when the unknown similarly appeared weak as the correct match point. This is the procedure which was advised by Haldane.

(c) To obviate the above inaccuracies, an observer should discover standard conditions of illumination and distance which enable him to compare the tints accurately, and use these conditions whenever he is determining the amount of haemoglobin in blood.

Summary

1. There was no difference in the accuracy with which ten trained and ten untrained observers matched the tints of solutions of carboxyhaemoglobin in daylight. The distance at which they made their comparisons was usually over 50 cm. and varied only within narrow limits in eighteen out of twenty observers.

2. The accuracy of seven out of ten untrained observers changed as the distance between their eyes and the comparison tubes changed.

3. Conditions of illumination and distance of comparison were found for seven out of ten untrained observers which enabled them to make accurate comparisons of tint.

4. The application of these observations to haemoglobinometry is discussed.

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RAPID AND ECONOMICAL RHESUS TESTING:
A TRIAL OF CHOWN'S CAPILLARY
TUBE METHOD

BY

GEORGE DISCOMBE AND H. MEYER

From the Central Middlesex County Hospital

Introduction

It is now firmly established that immunization by the Rhesus factor plays a predominant part in the pathogenesis of haemolytic disease of the newborn (Levine and others, 1941a and b), and that treatment by early induction of labour and transfusion or exsanguination transfusion will reduce the mortality of affected infants to about one quarter of that found in an untreated series (Diamond, 1947). It has also been shown that a single transfusion of Rhesus-positive blood will sensitize about half of all Rhesus-negative recipients (Moloney, 1945; Diamond, 1947; Hattersley, 1947), and that Rhesus-positive babies born to previously sensitized Rhesus-negative mothers are usually affected by a severe form of haemolytic disease. It is therefore essential to determine the Rhesus group of all female recipients of transfusions who are potential mothers, and to give only Rhesus-negative blood to Rhesus-negative recipients.

It is possible that the slide test, using the incomplete or blocking antibody (Diamond and Abelson, 1945), would be the method of choice, but unfortunately high-titre high-avidity sera of this type are not generally available in this country. Satisfactory sera derived from animals are troublesome to prepare. Sera of human origin containing the ordinary agglutinating antibody have thus to be used, but unfortunately sera with a high titre (1/32 or over), are seldom encountered, though there is less difficulty in obtaining sera with a titre of 1/8 or 1/16.

The standard technique, using precipitin tubes, is reliable but laborious; it also requires scrupulously clean glassware and relatively large volumes of sera. This report is concerned with a recent trial of the method of Chown (Chown and Lewis, 1944, 1946), to which our attention was directed by Dr. Race.

Method

In Chown's technique the cells to be tested are allowed to fall through a column of agglutinating serum contained in an inclined capillary tube of 0.3 to 1.0 mm. bore. If the cells are agglutinated they seem to become sticky and to adhere to the wall of the capillary in discrete clumps which can easily be seen by the naked eye, usually within ten minutes of starting incubation. Capillary tubes of about 0.5 mm. (0.3 mm. to 1.0 mm.) bore and about 7 cm. in length are required. These are easily drawn from 7 to 8 mm. diameter soft glass tubing and broken to approximate length between the fingers; several hundred may be made in half an hour. A block of plasticine is also needed, but if a large series is to be done it is convenient to replace the plasticine by a container of soft paraffin and to employ a perspex rack or the heated rack described by Chown and Lewis (1946) to hold the capillaries. An anti-D agglutinating serum from which the anti-A and anti-B agglutinins have been absorbed is employed.

A capillary is dipped vertically into the serum until a column 10 to 20 mm. long has entered by capillarity. It is then removed and dipped into the unknown test cell suspension until a column of about the same length has entered (care must be taken to avoid air-locks), inverted, and the lower end (which did not enter the fluids) stuck into the plasticine at an angle of about 45°: or, if a rack is used, the lower end is plugged with vaseline and the capillary placed in the rack. The capillary is then incubated at 38° C. and inspected at intervals. In about half of all Rhesus-positive samples, segmentation of the cell column will be visible in 5 to 10 minutes; this segmentation constitutes a positive reaction (Plate Ia). Most positives are definite in 15 minutes, but a few develop only after about

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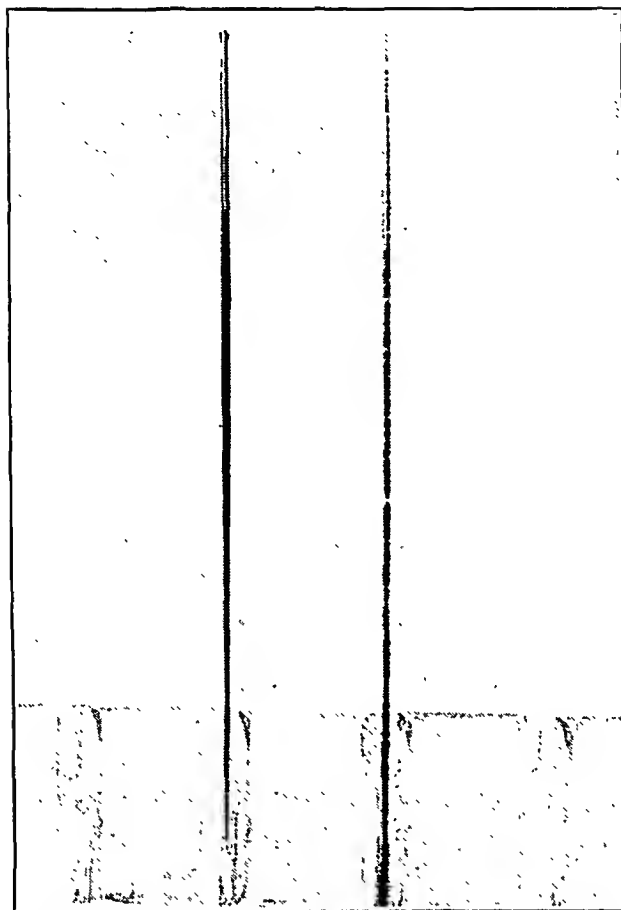
(a).—The capillary test. Left-hand tube—negative reaction. Right-hand tube—positive reaction.
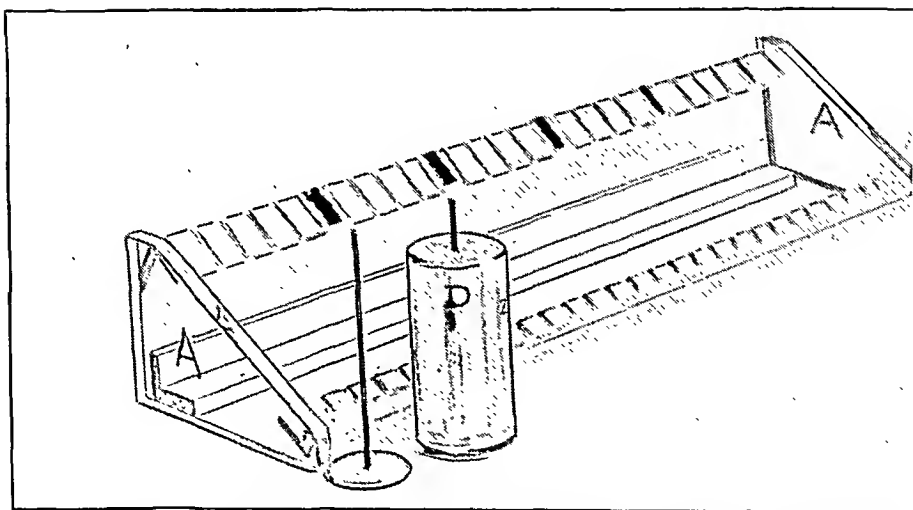
(b).—Perspex rack for tests. Polystyrene holder with handle for simultaneous washing of five samples of cells.

PLATE I.

30 minutes. A weak serum, or too dilute a cell suspension, delays the appearance of the positive reaction. If the cells are Rhesus-negative, there is no suggestion of segmentation, the cells forming a uniform smooth layer on the lower wall of the capillary. It will be noted that in cases of urgency, where the result has to be read within a short time, an occasional Rhesus-positive blood may be mis-read as negative (which is of minor importance), but never Rhesus-negative blood as positive.

The strength of the cell suspension is most im-portant. A mixture of fresh oxalated blood with once to twice its volume of isotonic citrate solution is recommended by Chown; this gives a cell suspension of about 15 per cent and is very satis-factory. However, most antenatal patients have blood taken for a Wassermann reaction. It was decided to use the residue of these samples, after the serum had been removed, by shaking the clot and the extruded cells with 1 to 2 volumes of saline. Such suspensions were found to be satis-factory, though suspensions made by removing the extruded cells by decantation, and shaking with fresh saline, were not, for they were always too dilute and frequently contained fragments of clot.

A comparison between this capillary method and the standard tube method has been made, a total of 503 specimens being examined. Of these, three were rejected owing to gross haemolysis or infec-tion, though two proved satisfactory in the tube technique; eight gave doubtful readings at the first trial of the capillary method, but became definite if a higher concentration of cells was used for the test; all these eight were over six days old. Alto-gether, there were 399 samples positive by both methods, of which 391 were clear-cut and obvious in the capillaries, and 101 samples negative.

On the basis of this comparison, it was decided that the capillary method was suitable for routine use, subject to certain limitations; first, the blood must be uninfected and not more than four days old; secondly, dilute suspensions of cells must be concentrated by centrifuging and resuspension; thirdly, doubtful reactions must be checked by the tube technique. The method has now been used for some months using anti-A, anti-B, anti-D, anti-C, anti-E, anti-c and anti-$(C+D+E)$ sera, and so far the results have been unexceptionable if fresh suspensions of cells at 10 to 20 per cent concentration have been used. It has also been used to demonstrate both complete and incomplete antibodies in unknown sera, but so far insufficient experience has been gained to warrant its general adoption for the detection of antibodies, though it appears to be at least as sensitive as the tube technique. Chown (1947) informs us that in his hands it is more sensitive, and we have succeeded in obtaining clear-cut results with one batch of serum so weak that it was useless for the tube technique.

A statistical analysis of the frequency of the D-antigen in (1) the 500 samples described, (2) a series of blood donors, (3) the first 400 antenatal patients studied as a routine, and (4) a series of 2,000 cases studied by Race (1947) has been made, the four series, and some of their combinations, being analysed for homogeneity by the x^2 method (Tables I and II).

TABLE I

Series	Rhesus-positive	Rhesus-negative	Total
1	399	101	500
2	124	35	159
3	341	59	400
4	1,658	342	2,000
Total	2,522	537	3,059

TABLE II

Comparison	x^2	P
1 with 2 with 3	6.27	0.04
1 with 2 with 3 with 4 ..	7.15	0.07
1 with 3	4.5	0.038
$(1 + 2 + 3)$ with 4 ..	0.848	0.62
1 with 4	2.6	0.1
3 with 4	2.75 .	0.1

It is obvious that there is a difference of doubtful significance between the series 1 and 3. Since all Rhesus-negative subjects are tested twice, once with anti-D and once with anti-$(C+D+E)$ serum, while many mothers of erythroblastotic infants have been transfused with blood from our own donors, it seems likely that the difference is due to chance.

Apparatus

If any number of tests are to be done, it is an advantage to have specially designed apparatus. We have used perspex racks similar to those advised by Berlin (1947). In our comparison of the two tech-niques we found that to avoid labelling tubes in

which cells were washed, blocks of translucent poly-styrene plastic could be used ; these were cylindrical, about 1¼ in. diameter and 2¼ in. long, with an axial hole to take a 20 S.W.G. brass wire. In each block five holes to carry precipitin tubes were drilled, each 2 in. long ; the block was labelled with a letter, and each hole for a tube with a number. Labelling is best done with an ink containing Sudan Black B dis-solved in a mixture of dichlorethylene and ethylene dichloride containing about 10 per cent Diakon (per-spex) cement. The block fits a 50-ml. centrifuge bucket, and can be lifted out by the wire handle. The wall of the block is so thin that the column of red cells in the tube is easily seen, and after centri-fuging the supernatant fluid can be sucked off and the cells resuspended without removing the tube from its holder (Plate I*b*). An apparatus for removal of supernatant fluids was also devised ; a suction pump is attached to a small Büchner flask, from which passes a rubber tube attached to a Pasteur pipette ; the lumen of the rubber tube is closed by a glass bead, and by pinching the rubber tube to one side of the bead a channel is formed.

Conclusion

The capillary method has been so satisfactory in our hands that we no longer use the tube method in routine Rhesus typing, but keep it for testing for agglutinins in unknown sera and for titrating sera. The capillary method requires little skill, the result usually being definite in fifteen minutes.

We are indebted to Dr. R. R. Race for drawing our attention to the method, to Dr. A. E. Mourant for supplying us with many of the antisera we have used, to the originator of the method, Dr. Bruce Chown, for his valuable advice, and to Mr. Tilman, assistant engineer to the hospital, for making apparatus to our design.

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APPENDIX

NOTE ON THE WORKING OF POLYSTYRENE ROD

BY

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The polystyrene plastic is brittle, and melts if machined carelessly or if it is not continuously cooled. Soap suds must be run over it continu-ously when machining or drilling. For turning, the rod is wrapped with a single layer of thick paper and held in a chuck and turned at 300 revolutions per minute. The tool must be of a good grade of tool steel, ground as for a side tool with nose slightly rounded off with a slight lip, and stoned to a fine edge ; ordinary grinding does not produce a smooth finish. Light cuts must be taken and coolant used continuously.

For drilling, a drilling machine or a lathe with instantaneous feed on a loose headstock must be used, since swarf chokes the flutes of the drill and leads to local melting. The drill is ground as for brass and honed to a keen edge ; it is fed lightly and cleared every 1/4 in. to 3/8 in., using a copious coolant.

As much material as possible should be left, especially where holes are close together.

A SIMPLIFIED PRICE-JONES TECHNIQUE*

BY

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AND

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The measurement of the mean diameter of the red blood cells is still one of the most important observations that can be made. Unfortunately the most accurate method of measurement by the technique introduced by Price-Jones is tedious and time-consuming. To trace the outlines of 500 cells, and then to measure the largest and smallest diameter of each, takes at least four hours. It also requires skill and practice. By the following technique, derived from that of Hynes and Martin (1936), and of Price-Jones as described fully by Mogenson (1938), the mean diameter of 500 cells can be measured and recorded in under one hour. Furthermore, the apparatus used is that found in all laboratories. It may be set up in four minutes.

Method and Material

The film is fixed with Leishman's stain for 1 min., rinsed with distilled water, and superstained with Field's eosin (Field, 1941) for 5 seconds. It is then dried in the air.

A " Pointolite " or similar lamp is used as a source of illumination. Any simple monocular microscope capable of being used in the horizontal position and fitted with a mechanical stage and oil immersion lens is used to project the image of the cells on to a series of rings drawn in indian ink on a white card mounted in a flat wooden base (Plate IIa). The rings are drawn with a fine-pointed compass with intervals of 1/2 mm. The range required is from 4.0 to 12 mm. A line 50 mm. long is also drawn on the card for calibration purposes.

The apparatus used should be set up in a darkened room on a horizontal bench which should be as flat as possible.

The mirror is removed from the microscope, which is placed in a horizontal position. The mirror is then held in a retort stand so as to reflect the light passing through the microscope on to the measuring rings laid on the bench (Plate IIb). The oil immersion lens is now used to adjust the magnification to ×1,000 by projecting the image of the side of a small square in the central ruled area of a thin Thoma-Zeiss haemocytometer (50 μ) on to the prepared card on its board. The haemocytometer grid should have been darkened by smearing on a little Leishman stain and allowing it to dry. The card on its board is adjusted on the bench so that the image of the square lies on the 50 mm. line. By altering the distance of the mirror from the object lens of the microscope the side of one square of the image is made to coincide with the 50 mm. line. The magnification is then ×1,000.

The stained blood film is now substituted for the haemocytometer and the image of the red cells is sharply focused on the card. By moving the card the cell diameters are measured by finding the ring which accurately contains the image of the cell. If the cell measured is not round, the ring chosen is that which is of such a size that the part outside the ring would fit into the gap left inside the ring.

In this way the diameters of as many cells as are required can be measured and recorded. The mean diameter and the standard deviation are calculated in the usual way.

Comment

The film should be as thin as possible and the part chosen for measurement should be where the red cells are not touching each other.

It is not necessary to measure 500 cells in every case, for, as Mogenson points out, the reduction

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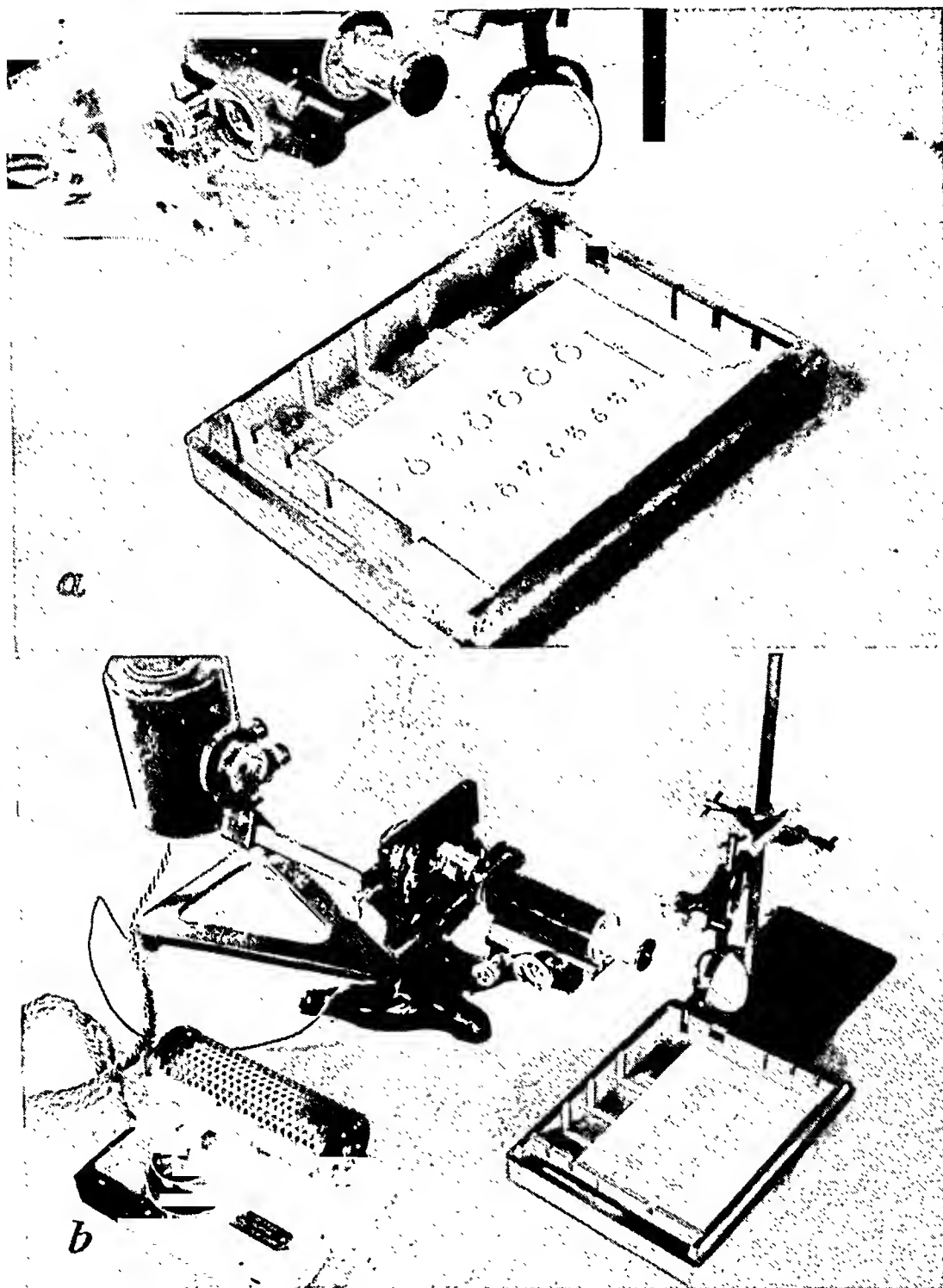
PLATE II.—(*a*) The card with the measured circles and 50 mm. line. (*b*) General view of the apparatus as set up.

of the standard error of the mean diameter obtained by increasing the number of cells examined from 200 to 500 is 7.2 per cent if the standard deviation is normal (up to 0.48) and only 19.2 per cent if the standard deviation is increased to 0.8.

The magnification should not be much increased above × 1,000, for the distinctness of the image decreases the further the light has to travel. The resolving power of the microscope also puts a limit to the magnification that can usefully be employed.

Summary

A simple method of obtaining Price-Jones curves quickly and economically is described.

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THE INHIBITION OF ACID PHOSPHATASES BY FORMALDEHYDE AND ITS CLINICAL APPLICATION FOR THE DETERMINATION OF SERUM ACID PHOSPHATASES

BY

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Introduction

The presence of considerable amounts of an active phosphatase with pH optimum about 5, normally present in human prostate, was first described by Kutscher and Wolbergs (1935). Many other tissues and fluids of the body contain acid phosphatases in variable amounts, but these are small in comparison with those found in the prostate and seminal fluid.

Normal plasma and serum contain small amounts (1 to 4 units) of acid phosphatase. It was first observed by Gutman and Gutman (1938) that the concentration of the enzyme increases in the sera of patients with metastatic carcinoma of the prostate, presumably due to neoplastic invasion of lymph or blood channels, with the escape of the enzyme into the circulatory fluids. In many instances the increases are so great as to be clearly diagnostic ; but in some cases there are only slight increases such as are occasionally found in other diseases. In such cases, therefore, a method for measuring the prostatic fraction of acid phosphatase is greatly needed.

On account of the marked susceptibility of the prostatic phosphatase to inactivation by certain narcotics, including alcohols, which was first reported by Kutscher and Wörner (1936), Herbert (1944, 1945, 1946) was able to devise a method for the specific estimation of prostatic phosphatase in serum. The basis of Herbert's method is that incubation of serum or plasma with (two-fifths volume) absolute alcohol at room temperature for 30 minutes completely destroys the prostate enzyme fraction but hardly affects the normal plasma acid phosphatase.

During the course of a careful study of this method we have tried the effect of alcohol incubation on serum acid phosphatase in a great number of cases of normal and non-prostate diseased persons. We have found that, while in many cases the serum acid phosphatase is not significantly affected by this alcohol treatment, yet in a good number of cases a marked inhibition is observed, for example in certain types of liver disorders and other diseases. In other cases the alcohol treatment leads to an actual, and unexplained, increase in the total acid phosphatase (cf. Appendix).

This directed our attention to the possibility that this lability of the prostatic enzyme to the destructive action of alcohol may be shared by other tissue phosphatases as well. It has been found that while many tissue acid phosphatases, including those of the adrenals, intestines, liver, spleen, etc., are not affected by alcohol incubation, the red cells, bile, and kidney enzymes are, on the other hand, seriously inhibited.

The red blood corpuscles are second to the prostate in regard to their high content of acid phosphatase, and contain nearly one hundred times the amount present in plasma. King and others (1945) found the acid phosphatase of prostate and red cells to be similar in many respects, including easy destruction by alcohol.

Thus, for applying Herbert's technique, the sera should be strictly free from any trace of haemolysis. This introduces great difficulty as it has been found in practice that the majority of samples of blood taken in the wards for analysis show variable degrees of haemolysis. Even when blood is taken with great care, unless the plasma is

separated as soon as possible, there is always the possibility of escape of the enzyme from the red cells into the plasma, even without detectable haemolysis. This has been found especially in cases when the corpuscles are abnormally rich in acid phosphatase. Many samples sent to the laboratory showing apparent haemolysis have to be reported " unfit for acid phosphatase determination."

For the above reasons it is felt that there is a distinct need for a more reliable method, sufficiently simple to be adopted in routine work, giving results of definite diagnostic values in prostatic cases, and not influenced by the corpuscular or other body tissue enzymes other than those of the prostate.

It has been found, after trying a series of organic substances belonging to many different groups, that formaldehyde answers many of these requirements. Under our experimental conditions, formaldehyde destroys completely the red-cell enzyme while not affecting the prostate enzyme to any appreciable degree. Other tissue acid phosphatases are variably inhibited by formaldehyde, but none is found to be so sensitive to this reagent as the corpuscular enzyme.

The normal serum or plasma acid phosphatases are also seriously damaged by formaldehyde. Thus, by applying a very simple formaldehyde technique, we can always get the prostatic fraction of serum acid phosphatase free, or nearly free, from the other interfering acid phosphatases.

Methods

The King and Armstrong (1934) phenyl phosphate method, as modified by Gutman and Gutman (1938, 1941) for the estimation of serum acid phosphatase, has been followed in principle. Certain modifications are introduced to suit either Herbert's technique or the proposed formaldehyde procedure, both of which are described here in detail.

Reagents

Acid citrate buffer.—*p*H 4.9. Disodium citrate, 21.25 g., dissolved in 1 l. distilled water containing 100 ml. N/10 HCl. The solution should be preserved with 5 ml. chloroform and kept in the cold.

Substrate.—M/50 disodium phenyl phosphate*: 2.18 g. dissolved in 500 ml. distilled water. The solution should be brought quickly to the boil to destroy any organisms, cooled immediately, and preserved with 5 ml. chloroform and kept in the cold.

* Commercial specimens of disodium phenyl phosphate may contain traces of free phenol, which add appreciably to the blank colour in the phosphatase method. Any free phenol is easily got rid of by washing the solid disodium phenyl phosphate with ether, after which it should be dried in the air.

Folin-Ciocalteu phenol reagent (1927).—Diluted 1 in 3.

25 per cent sodium carbonate (anhydrous) (w/v). —This solution should be kept in a warm place.

Standard-phenol-and-reagent. — (1 mg. phenol/ 100 ml.) 5 ml. of stock standard phenol (100 mg./ 100 ml.) is accurately measured into a 500-ml. volumetric flask ; 100 ml. of dilute (1 :3) Folin-Ciocalteu reagent is added, and water to the mark.

Formaldehyde reagent.—(2 per cent formaldehyde solution.) 5 ml. of neutral formaline solution (40 per cent) is pipetted into a 100-ml. measuring flask and diluted to the mark with distilled water.

Procedures

Fresh serum or plasma must be used, and examined with the visual spectroscope, if necessary, to be sure of complete absence of haemolysis whenever the alcohol procedure is to be adopted.

Total acid phosphatase.—1 ml. substrate and 1 ml. distilled water are pipetted into each of two centrifuge tubes together with 2 ml. of the buffer solution. The tubes are allowed to remain in a 37° water-bath for three minutes to allow the contents to attain the temperature of the bath. After this time 0.2 ml. of the serum is added to one of the tubes and the enzyme hydrolysis allowed to proceed for exactly one hour, at the end of which time 1.8 ml. of Folin and Ciocalteu's phenol reagent are added to each tube and 0.2 ml. of serum to the second tube. The two tubes are shaken and centrifuged, and 4 ml. of the supernatant pipetted into two test tubes. After the addition of 1 ml. of 25 per cent sodium carbonate solution, the tubes are replaced in the water-bath for fifteen minutes to allow the colours to develop. The colour intensity is compared with that of a standard solution of phenol (i.e., 4 ml. of standard-phenol-and-reagent and 1 ml. of sodium carbonate) which has been similarly treated. A photo-electric colorimeter is used for this purpose, setting its zero with water, or, better, a blank of 3 ml. water, 1 ml. phenol reagent, and 1 ml. sodium carbonate solution (without serum), and using a red filter. The difference between the incubated and non-incubated samples gives the phenol formed by hydrolysis. A unit of acid phosphatase is defined as the amount of enzyme which will liberate 1 mg. phenol per 100 ml. serum or plasma per hour from M/200 disodium phenyl phosphate substrate at a *p*H near 5 at 37° C. (If the colours are too deep for reading in the available colorimeter, the coloured solutions (test, control, blank, and standard) should be diluted to 10 ml. with water.)

Calculation :

$$\left(\frac{T}{S} \times 0.04 \times \frac{6}{4} \times \frac{100, \text{ for}}{0.2 \text{ test}}\right) - \left(\frac{T}{S} \times 0.04 \times \frac{6}{4} \times \frac{100, \text{ for}}{2 \text{ control}}\right)$$

$$= \left(\frac{T}{S} \times 30, \text{ for} \atop \text{test}\right) - \left(\frac{T}{S} \times 30, \text{ for} \atop \text{control}\right) = \text{units phosphatase}/100 \text{ ml.;}$$

where T=reading of test ; S=reading of standard ; 0.04 = mg. phenol in the 4 ml. of standard-phenol-and-reagent ; 6=the vol. of 1 ml. substrate ÷ 1 ml.

G

water + 2 ml. buffer + 0.2 ml. serum + 1.8 ml. Folin-Ciocalteu ; 4 = the vol. of protein-free supernatant taken ; and 0.2 = the vol. of serum.

Alcohol inactivation.—To 1 ml. of serum or plasma, 0.4 ml. of absolute ethyl alcohol is added. After shaking, the tube is stoppered and the mixture allowed to stand for half an hour at room temperature. Water (0.6 ml.) is added, and a sample of 0.4 ml. of the opalescent mixture (corresponding to 0.2 ml. of serum or plasma) is added to the warm, previously mixed, 2 ml. buffer + 1.0 ml. substrate and 0.8 ml. water to make a final volume of 4.2 ml. buffer-substrate-enzyme mixture. The procedure is then followed exactly as above for total acid phosphatase.

Formaldehyde technique.—The procedure is exactly as described above under " total acid phosphatase " except that, in this case, 1 ml. formaldehyde reagent is added instead of the 1 ml. distilled water to the mixture of 2 ml. buffer and 1 ml. substrate solutions. All the following details are then the same.

Experimental

THE RELATIVE CONTENT OF " ACID " AND " ALKALINE " PHOSPHATASES IN SOME HUMAN TISSUES

Since we are mainly concerned with the serum acid phosphatases, we thought it necessary to examine the various body tissues which are believed to be responsible for the variation of this enzyme in plasma in health and disease.

Although a good deal of work described in the literature has been carried out on various animal tissues in this connexion, very little has actually been done on human material. Normal adult tissues, without any apparent disease, were obtained from autopsy within 24 hours of death. After being freed from fat and other adhering extraneous tissue, they were carefully washed with normal saline and then with cold tap water until they were as free as possible from any blood cells. They were then dried by filter paper, weighed and ground with sand, and mixed intimately in a mortar with an equal weight of normal saline solution and 10 per cent (by volume) of a mixture of equal parts of toluene and ethyl acetate. After having been left to autolyse at room temperature for two or three days with occasional shaking, the mixtures were centrifuged and the supernatant liquids filtered clear through a Whatman No. 1 folded filter paper.

Tissue extracts obtained in this way are always clear and free from any colour of blood, and their enzyme activity corresponds to that present in the original wet tissue. They keep their activity unchanged for several weeks if kept in the ice chest. They should be diluted ten to twenty times, or even more in certain cases, with normal saline just before their activity is determined. In the case of the red cells, the freshly drawn blood was directly centrifuged, the plasma separated, and the red cells washed twice with normal saline by spinning and decantation. 1 ml. of washed red cells was lysed in 9 ml. water and the solution centrifuged. The clear supernatant was used directly for determining its enzyme activity. The prostate gland was decapsulated, freed from adhering tissue, cut into fine pieces, and ground with sand in a mortar with five times its weight of normal saline solution. A few drops of toluene were added but no ethyl acetate. The mixture was left at room temperature to autolyse for two or three days with occasional gentle rotating. (Vigorous shaking should be avoided as it may inactivate the enzyme.) The mixture was then filtered through filter paper. It was highly diluted (e.g.—1,000 to 5,000 times) before determining its enzyme activity.

TABLE I

ALKALINE AND ACID PHOSPHATASE OF HUMAN TISSUES

Tissue	No. of specimens examined	mg. phenol liberated/hr./1 g. wet weight tissue				Ratio alkaline/ acid
		Alkaline phosphatase		Acid phosphatase		
		Range	Average	Range	Average	
Adrenal glands	6	25–38	30	2–5.5	3.0	10
Bile from gall-bladder	5	10–23	16	0.3–7.0	2.5	6.2
Intestinal tissue	5	49–72	53	2–3	2.7	20
Kidney	6	12–24	16	6.5–18	10	1.6
Liver	6	12–37	14	7–13	9.7	1.4
Pancreas	5	0–6	3	3–11	5.7	0.5
Prostate	10	0.1–1.2	0.5	2,000–10,000	4,000	0.00025
Red cells	20	0.6–2.5	1.5	8–25	15	0.1
Spleen	5	10–17	14	4.5–8.5	6.7	2.0
Thyroid	4	2–4	3	1.5–3	2.0	1.5

The phosphatase activity of these preparations was determined at two hydrogen ion concentrations; namely, pH 5 and pH 10, using the above-described acid citrate buffer for the first, and alkaline carbonate-bicarbonate buffer (Delory and King, 1945) for the latter, with disodium phenyl phosphate as substrate. For the sake of comparative. study of the relative activity of the two enzymes at these two pH values, the buffer–substrate–enzyme mixtures were incubated for 60 minutes at 37° C. The phenol liberated at the end of that time was taken as an index of the enzyme activity at the corresponding pH.

Thus Table I gives the results expressed in mg. phenol liberated per 1 g. wet tissue per hour at the two given pH values. (No Mg was added.)

Table I does not include the bone phosphatases, which, in the normal adult, are not expected to be of much activity. The intestines are the richest human source of alkaline phosphatase, followed by the suprarenals. The human kidney seems to be very poor in alkaline phosphatase compared with other animals' kidneys, while the human liver appears to contain much more alkaline enzyme than that of other animals.

As for the acid phosphatases, the prostate is unquestionably the richest source in the human. The only other animal in which it occurs in large amounts is the monkey and then only in the caudal lobe of the prostate, as shown by Gutman and Gutman (1939). The second tissue that is rich in acid enzyme is the red cell, although the difference is very great. Red cell phosphatase varies enormously in different individuals, for no known reason, but generally speaking it has been found to diminish in activity with age.

The human kidney, liver, pancreas, and spleen contain appreciable amounts of acid phosphatase.

EFFECT OF ALCOHOL INCUBATION AND FORMALDEHYDE TREATMENT ON DIFFERENT TISSUE ACID PHOSPHATASES

The tissue extracts described above were subjected to both alcohol incubation and formaldehyde treatment in the same way as described for serum. Table II shows the result of several experiments carried out in this connexion on different tissue extracts.

From Table II the inhibitory action of alcohol on prostate, bile, kidney, and red cells is evident.

The failure to achieve 100 per cent inactivation of the prostate enzyme by alcohol incubation can be explained by the fact that we were working with enzyme solutions in normal saline. Herbert showed that certain conditions must be fulfilled in

TABLE II

EFFECT OF ALCOHOL AND OF FORMALDEHYDE ON ACID PHOSPHATASES

Tissue preparation	Acid phosphatase units/ 100 ml.*	Percentage inhibition	
		After alcohol	With formaldehyde
Adrenals ..	21–64	0	12–25
Bile ..	1.5–34	80–100	20–50
Intestines ..	35–71	0	40–50
Kidneys ..	37–153	17–62	24–50
Liver ..	35–92	0	30–60
Pancreas ..	30–60	0	15–40
Prostate ..	25–300	40–70	0
Red cells ..	9–120	40–55	100
Spleen ..	17–127	0	44–52
Thyroid ..	20–75	0	25–40

* These figures represent the enzyme activities of extracts of tissues at various dilutions prepared to give a convenient concentration of phosphatase for accurate measurement. They do not represent the comparative enzyme contents of the tissues themselves.

order to get complete inactivation of the prostate enzyme solution with alcohol incubation : for example, the presence of a sufficient bicarbonate concentration and other serum salts of weak acids and strong bases is essential.

The destruction of the bile acid phosphatase with alcohol is especially striking, since that of the liver is not affected. It might be possible that this inhibition is indirectly due to certain other bile constituents affected by the alcohol incubation. Until both the liver and the bile enzymes are prepared in a purer state, we cannot decide if there is a real difference between them.

Formaldehyde inhibits the different tissue enzymes to variable extents (20 to 60 per cent), but its 100 per cent inhibitory action on red cells is remarkable : likewise its complete lack of any effect on the prostatic phosphatase.

The difference between the red-cell enzyme on the one hand, and the liver and spleen enzymes on the other, which was first described by Davies (1934), is further shown here in regard to their behaviour towards alcohol and formaldehyde.

EFFECT OF ALCOHOL INCUBATION AND FORMALDEHYDE TREATMENT ON NORMAL PLASMA AND ON PROSTATE AND RED CELL SOLUTIONS IN PLASMA

The following illustrations of the effect of alcohol incubation and formaldehyde treatment on normal plasmas alone, and on those to which prostate extracts and red-cell solutions have been added, form the basis for the clinical application of these methods.

It has been shown by Herbert that in the case of alcohol incubation the behaviour of the prostate enzyme solution in normal saline differs from that in serum or plasma. It is more strongly inhibited when present in the latter state than when it is merely in saline solution. Thus the question arises as to whether the prostate and red-cell enzyme solutions in water will behave differently to those in serum or plasma with regard to the formaldehyde treatment. The answer is found in Table III, from which it is seen that in plasma or serum media the formaldehyde still exhibits its strong inhibitory

action on the red-cell enzyme while not affecting at all the prostate phosphatase.

Normal plasma phosphatases are sensitive to formaldehyde but resistant to alcohol. The alcohol incubation seems to destroy totally the prostate enzyme in serum or plasma medium, while the red-cell enzyme is only partly destroyed.

The formaldehyde, however, provides a quantitative means for estimating the prostate enzyme in the presence of the red cell. This is clearly shown in Table IV, in which two solutions, one of prostate extract and the other of red cells, both of known enzyme activity, are mixed together in different proportions and subjected to formaldehyde treatment. The residual values after formaldehyde treatment always agree closely with those corresponding to the amount of prostate enzyme added. Table IV shows that a quantitative determination

TABLE III

EFFECT OF ALCOHOL AND FORMALDEHYDE ON PLASMA PHOSPHATASE ENRICHED BY THE ADDITION OF PROSTATE AND RED-CELL PHOSPHATASES

	Acid phosphatase units/ 100 ml.	After alcohol incubation	After formaldehyde treatment
1. Normal plasma	4.5	4.0	1.1
,, ,, + prostate ext.	48.6	6.3	45.0
,, ,, + prostate ext.	73.5	3.7	69.7
,, ,, + red cells soln.	45.7	17.3	1.5
2. Normal plasma	6.0	5.2	1.5
,, ,, + prostate ext.	100.5	6.7	95.2
,, ,, + red cells soln.	37.5	24.0	3.7
,, ,, + red cells soln.	66.0	15.0	3.2
3. Normal serum	3.0	4.0	1.5
,, ,, + prostate ext.	48.0	3.0	47.2
,, ,, + red cells soln.	24.0	13.2	1.5
4. Plasma	3.5	4.6	1.5
,, + prostate ext.	49.5	4.5	47.5
,, + red cells	31.5	10.5	1.5

TABLE IV

AGREEMENT BETWEEN THE CALCULATED AND DETERMINED ACID PHOSPHATASE CONTENTS OF PROSTATE–RED-CELL MIXTURES

	Units/100 ml. solution		Calculated units of prostate enzyme in mixture
	Without formaldehyde	With formaldehyde	
Solution (A) prostate ext.	93	93	
Solution (B) blood cells	30	0	
Mixture of (A) + (B)			
1(A) + 1(B)	64.5	48.0	46.5
1(A) + 2(B)	51.5	31.5	31.0
2(A) + 1(B)	73.0	62.0	62.0

TABLE V

INHIBITING EFFECT OF VARIOUS CONCENTRATIONS OF FORMALDEHYDE ON TISSUE ACID PHOSPHATASES

	Units/100 ml. before treatment	Units/100 ml. after treatment with formaldehyde			
		1%	0.5%	0.25%	0.1%
Normal serum	4.3	1.5	1.5	3.3	3.7
Prostate extract	150.0	140.0	148.5	150.0	150.0
Normal serum + prostate	45.5	39.8	42.5	43.5	44.0
Red cells in water	40.0	0	0	0	0
,, ,, ,, ,,	140.0	0	0	12.0	15.0
Normal serum + red cells	24.0	1.5	1.5	3.0	3.5
Adrenals	21.0	16.5	—	—	21.0
Kidney extract	38.6	20.5	21.5	22.6	24.6
Liver ,,	35.5	18.7	21.4	22.0	23.6
Pancreas ,,	13.5	11.2	11.2	—	11.2
Spleen ,,	56.2	24.1	26.8	27.5	—

of prostate and red-cell enzymes, when present in a mixture, can be easily effected.

EFFECT OF VARIOUS CONCENTRATIONS OF FORMALDEHYDE ON DIFFERENT ACID PHOSPHATASES

The concentration (final) of formaldehyde, so far used in all the previous experiments, and which is suggested for use in routine work, is 0.5 per cent in the enzyme–substrate–buffer mixture.

This concentration has been found experimentally to be the maximum that can be used without affecting the prostate enzyme, while producing its maximum inhibition of the serum acid phosphatases. Table V shows the effect of various concentrations of formaldehyde. The extreme sensitivity of the red-cell enzyme to formaldehyde is apparent. As little as 0.1 per cent of this reagent completely destroys small amounts of the enzyme, while inhibiting nearly 90 per cent red-cell preparations of high phosphatase activity (for example, 140 units/100 ml.). On the other hand solutions of formaldehyde stronger than 0.5 per cent affect the prostate enzyme. The plasma and serum acid phosphatases seem not to be inhibited beyond certain limits even with increase of formaldehyde concentration. The 0,5 per cent concentration of formaldehyde seems, therefore, to be just suitable for the purpose.

CLINICAL APPLICATION

The alcohol and formaldehyde techniques have been applied to a variety of plasmas and sera from different types of disease, covering 67 prostate cases, 24 cases of other tissue cancers, 15 cases of bone disease, 38 cases of liver disease, 16 cases of kidney disease, and 56 cases of other miscellaneous diseases.

The majority of these samples were subjected to both formaldehyde and alcohol incubation procedures, except in some instances where there was either shortage of material or where the samples showed slight signs of haemolysis. The majority of samples were collected from the Hammersmith Hospital, others from other London hospitals, and the sera and plasmas, which were separated as soon as the blood was received, were kept in the ice chest until the tests could be carried out (usually without delay).

Results and Discussion

A synopsis of the results of the clinical application of both alcohol incubation and the formaldehyde method to pathological sera and plasma is given in Table VI and the results appear in full in the appendix.

For convenience the "total acid phosphatase" figures are classified into two main groups: "normal values" (those up to 5 units); and "abnormal values" (more than 5 units per 100 ml.).

TABLE VI

SUMMARY OF ACID PHOSPHATASE RESULTS

(Total acid phosphatase units in roman type, formaldehyde-stable in italics)

Diagnosis	0 to 1.5	1.6 to 3.	3.1 to 5	5.1 to 7	7.1 to 10	10.1 to 15	15.2 to 20	21 to 30	31 to 40	41 to 50	51 to 70	71 to 100	>100
Carcinoma of prostate with bone metastases (22)*	—	2	4	1	1	2	4	2	—	2	—	—	4
	4	2	2	—	3	2	2	1	1	1	—	—	4
Carcinoma of prostate; no bone metastases demonstrable (22)	—	4	6	4	4	3	—	—	—	—	—	—	1
	5	9	5	1	—	1	—	—	—	—	—	—	1
Senile hypertrophy, and retention with enlarged prostate (23)	—	9	4	3	2	—	3	2	—	—	—	—	—
	8	8	1	2	2	—	1	1	—	—	—	—	—
Cancer of organs other than prostate (24)	—	3	8	5	4	4	—	—	—	—	—	—	—
	9	12	3	—	—	—	—	—	—	—	—	—	—
Paget's disease of bone (15)	—	4	5	4	1	1	—	—	—	—	—	—	—
	11	1	2	1	—	—	—	—	—	—	—	—	—
Obstructive jaundice (18)	1	2	2	7	4	1	1	—	—	—	—	—	—
	2	7	6	2	1	—	—	—	—	—	—	—	—
Other liver disease (hepatitis, cirrhosis, etc.) (20)	—	7	5	1	4	1	1	1	—	—	—	—	—
	7	6	5	2	—	—	—	—	—	—	—	—	—
Nephritis (16)	2	7	1	3	1	2	—	—	—	—	—	—	—
	7	4	3	2	—	—	—	—	—	—	—	—	—
Miscellaneous (56)	5	18	16	6	3	3	3	2	—	—	—	—	—
	29	20	3	4	—	—	—	—	—	—	—	—	—

* Number of cases.

PROSTATE CASES

The prostate cases examined, although not great in number, furnish a useful conclusion as to the successful clinical application of the formaldehyde technique. These cases are as follows:

Cases of carcinoma of prostate with bone metastases.—These are 22 in number; 16 of them gave abnormal values (>5) for the total acid phosphatase (73 per cent). Compared with the latest published results, Herbert (1946) found that about 83 per cent of 35 cases of prostatic cancer showing bone secondaries gave acid phosphatase values above 5, while Woodard and Dean (1947) found 72 per cent of 71 cases examined to give abnormal values.

Considering the formaldehyde-stable fraction in cases examined in the present work, it is noted that 16 cases out of the 22 (73 per cent) gave values above 3 units, while 14 of the latter gave values above 5 units. This means that if we adopt the figure 3 as the maximum permissible normal value for the formaldehyde-stable serum acid phosphatase (see below), we still get the same percentage of abnormal values.

The formaldehyde-stable fraction in most abnormal cases coincides fairly well with the alcohol-labile fraction, that is, with the difference between the total acid phosphatase and the alcohol-stable fraction (except in a few cases where the alcohol-stable fractions were unexpectedly high). If the two figures (after both alcohol incubation and formaldehyde treatment) be added together, values very near to the original total acid phosphatase are obtained (see Appendix).

The formaldehyde procedure provides a more reliable means of following the progress of cases under oestrogenic treatment than does the total acid phosphatase. High "total" values have shown a pronounced tendency to fluctuation, whereas the formaldehyde-stable figures steadily decreased during the course of favourable responses to treatment.

Cases of carcinoma of prostate without bone metastases (as shown by histological and radiological examination).—These are 22 in number. In 12 of them (55 per cent) the total acid phosphatase figures are above normal (i.e., >5); while with formaldehyde only 7 (32 per cent) gave values above 3 units and only 3 (14 per cent) gave values above 5 units. In one case only was the acid phosphatase very high (>100): repeated radiological examination failed to reveal any evidence of bone secondaries, until, in the latest film, an apparent abnormality was noted in one of the lumbar vertebrae.

Other prostate cases.—These comprise 23 cases of adenoma, acute retention with enlarged prostate, and senile hypertrophy. Most of these cases were examined radiographically and no evidence of bone lesion was found. The prostate growths were found histologically to be benign.

Ten cases (45 per cent) showed abnormal total acid phosphatase values while 7 (32 per cent) gave formaldehyde-stable figures above 3 units, and 6 (27 per cent) gave values above 5 units.

NON-PROSTATIC CASES

Cancer of various organs.—In this group figures are given for 24 cases of cancer of different organs other than the prostate. In some of these there were secondaries in the bones, and the akaline phosphatase in one case is very high (85 units). In many of the cases there is a tendency to an increased total acid phosphatase value, not necessarily, however, paralleled by an increase in the akaline phosphatase: for example, the highest total acid phosphatase value (15 units obtained in a case of cancer of bone) was accompanied by an akaline phosphatase of only 23 units; while the case of carcinoma of the bronchus, invading several organs including bone, with the very high alkaline phosphatase (85 units), had only 3.4 total acid phosphatase units.

Of the 24 cases examined, 13 gave abnormal total acid phosphatase values (that is, above 5 units; about 54 per cent). With the formaldehyde technique none gave values above 5 units, and only 3 cases (about 12 per cent) gave values above 3, the highest of them being only 4 units.

Paget's disease of bone.—Fifteen cases of bone disease were studied. All of them were Paget's. Five are moderately high and one high. In Paget's disease there is no parallelism between the total acid phosphatase and the increased alkaline phosphatase values.

It is known that the phosphatase of bone is very sensitive to an acid pH, and one would not, therefore, expect any activity on the acid side of neutrality from bone phosphatase preparations, however high their activity might be on the alkaline side. Consequently, any increase in acid phosphatase in bone disease should be considered of non-osseous origin. In secondary carcinoma of bone, with the primary growth in organs other than the prostate, there might be a tendency, however, to high total acid phosphatase values. This was found to be the case in two sera examined. But formaldehyde treatment reduced all values obtained in bone disease to a range from 0 to about 3 (except in one Paget's, where it was 5.5).

Some authors are of the opinion that an extremely high serum alkaline phosphatase derived from bone may show. its activity slightly at the acid reaction. But formaldehyde will exclude this completely, as the alkaline bone enzyme is very sensitive to this reagent. In the one Paget's case, with an alkaline phosphatase of 140, and a formaldehyde resistant phosphatase of 5.5 (out of a total acid phosphatase of 7.5), the 5.5 value cannot be taken as a mere reflection of the very high alkaline phosphatase of bone origin. Such elevated phosphatases must be of non-osseous origin.

The possibility of the local production of acid phosphatases in tumours and abnormal active growths should not be neglected. Gutman and others (1936) observed that, in addition to alkaline phosphatase, acid phosphatase was markedly increased at the site of osteoplastic metatases secondary to carcinoma of the prostate gland. This was confirmed by Barringer and Woodard (1938). This acid phosphatase may be different from that of the prostate since it seems to be formaldehyde labile in most cases, as can be judged from the high total acid phosphatase levels, compared with the comparatively small formaldehyde-stable values, in cases of carcinoma of the prostate with bone metastases during treatment with oestrogens.

Liver disease.—The most striking incidence of high values of non-prostatic acid phosphatases occurs in liver diseases. 21 cases out of the 38 studied gave raised total values above 5 units. As has been previously pointed out, the bile acid phosphatase, which might account for some of the high values found in jaundice and other liver disease, is completely destroyed by the alcohol incubation and partially by formaldehyde. On the other hand the liver acid phosphatase is not attacked by alcohol and only partly by formaldehyde. The results of alcohol incubation and formaldehyde treatment, shown in the table, suggest that the abnormal amount of acid phosphatase present is derived both from the liver and from the bile, since it is only partly destroyed by both alcohol and formaldehyde in most cases.

Although formaldehyde treatment does not furnish a complete differentiation in cases of liver disease which show a tendency towards elevated formaldehyde-stable values (reaching 7.5 in 1 case), nevertheless it is still the most useful indicator we have (although some other means of sharper differentiation may eventually be found).

Miscellaneous diseases.—The result of applying the inactivation methods to the serum acid phosphatases in a variety of other diseases indicates that there is a tendency for an increased acid phosphatase in some kidney cases. The alcohol incubation method affected quite a number of them. In a total of 53 cases (all free from haemolysis) subjected to the alcohol incubation method, 13 showed reduction of more than 1 unit.

As regards the formaldehyde treatment, it is noted that out of the total of 72 cases examined by this technique, 60 (i.e., 84 per cent) of them gave formaldehyde-resistant values between 0 and 3 units, while 6 (8 per cent) gave figures of 3 to 5, and 6 above 5 units.

It is striking that in some cases, for example, of anaemia, there is a small total acid phosphatase which is totally resistant to formaldehyde. This is not necessarily to be taken as of prostatic origin, for it occurs in both male and female cases. There may be present in some tissues not yet shown an acid phosphatase which is similar to the prostate enzyme in its stability towards formaldehyde, but which is not destroyed by alcohol.

Conclusion

The value of 5 units of total acid phosphatase, arbitrarily agreed upon by several workers as the maximum (normal figure) for non-prostate cases, has been exceeded in a good number of non-prostate cases. It is desirable to have a method giving a more restricted range in non-prostatic cases while not affecting the prostatic acid phosphatase. This has been satisfactorily found in the proposed formaldehyde technique.

Although the number of cases examined (216 in all) is not great, yet we believe we have sufficient data to support the following suggested limits for the formaldehyde-stable acid phosphatase:

Normal values: from 0–3 units per 100 ml. serum or plasma.

"Suspicious": from 3.1–5 units per 100 ml. serum or plasma.

Prostatic: above 5 units per 100 ml. serum or plasma.

In most cases the simple formaldehyde treatment will serve to distinguish between the high acid phosphatase of prostatic origin and those accompanying other conditions. In a few cases this will not suffice, and Miss Herbert's technique of alcohol treatment should then be employed. But experience seems to show that certain liver cases (and possibly some cases of bone disease) are the only instances where confusion with prostatic disease is likely to arise, and here an alkaline phosphatase, together with other characteristic chemical and clinical tests, should resolve any ambiguity

In addition to being useful for the study of special and difficult cases, formaldehyde and alcohol have a definite utility, along with other inhibitors and activators, in attempts to differentiate the several acid phosphatases of the different tissues of the body. We have. evidence, for instance, of at least 2 acid phosphatases in most tissues and of there only being one in the prostate.

Summary

1. A study of the relative content of the acid and alkaline phosphatases of some human tissues has been made.

2. The effect of alcohol incubation was tried on these tissue acid phosphatases, and it has been shown that while those of the adrenals, intestine, liver, pancreas, spleen, and thyroid are not affected, those of the bile, kidney, red cells, and prostate, on the other hand, are seriously inhibited.

3. The effect of formaldehyde on the acid phosphatases of prostate and other tissues, including the red cells, was studied under certain conditions. Its complete destructive action on the red-cell enzyme, and its lack of any effect on the prostate enzyme under the same conditions, are reported. The other tissue acid phosphatases are variably inhibited, but none is similar to the red-cell enzyme in being completely inactivated.

4. The introduction of a formaldehyde technique in routine determinations of the serum or plasma acid phosphatase is suggested, and a method described.

5. The alcohol incubation method and the formaldehyde technique were applied to 216 pathological sera and plasmas, including prostate cases and other cases of a wide variety of diseases. The results are discussed, and limits for the formaldehyde-stable fraction of serum acid phosphatase, for what should probably be considered non-prostate, are suggested.

6. A high acid phosphatase after formaldehyde treatment (above 5 units) strongly suggests a prostatic origin (values >3 are " suspicious "), and in most cases it gives useful information for distinguishing between raised values due to the presence of prostatic phosphatase, and those of different origin. An alcohol incubation test gives further confirmatory evidence in doubtful cases.

7. The formaldehyde technique permits the use of haemolysed sera or plasmas which are otherwise unfit for acid phosphatase determination.

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APPENDIX

PLASMA ACID PHOSPHATASE (TOTAL, ALCOHOL-STABLE, AND FORMALDEHYDE-STABLE) IN PATHOLOGICAL SERA

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol-stable	Formaldehyde-stable		
PROSTATE CASES (67) Carcinoma of prostate with bone metastases (22)					
14.1.47	—	—	—	—	Admitted to hospital. Very ill
20.1.47	119	19	119	127	10 mg. oestradiol (i.m.)+20 mg.stilb-oestrol (O.) daily for 8 days
29.1.47	55	5.8	49	120	Little improvement
4.2.47	39	—	33	128	40 mg. stilboestrol daily for 10 weeks
26.2.47	17	—	11	86	Improvement
3.3.47	17	—	12	76	
10.3.47	17	—	11	142	
14.3.47	23	—	6.0	180	
17.3.47	13	—	0	150	
19.3.47	9.0	—	1.5	118	Intermittent oedema
23.3.47	9.0	—	4.5	105	
26.3.47	7.5	—	3.0	58	Condition eased
2.4.47	8.2	—	3.7	65	
9.4.47	3.0	—	1.8	44	

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/ 100 ml.)	Notes
	Total	Alcohol-stable	Formaldehyde-stable		
PROSTATE CASES (67) *(continued)* 1 *(contd.).* Carcinoma of prostate with bone metastases (22) *(contd.).*					
17.4.47	22	—	6.6	48	Liver enlarged
24.4.47	14	—	7.3	43	200 mg. stilboestrol orally daily for 30 weeks
1.5.47	17	22	5.1	40	
8.5.47	21	23	5.1	43	
12.5.47	8.2	8.0	2.1	39	
15.5.47	9.2	10	3.7	38	
22.5.47	12	—	3.5	36	
29.5.47	13	—	5.0	36	
5.6.47	11	—	3.0	45	
12.7.47	4.7	3.7	2.2	33	
23.8.47	—	—	—	—	Discharged on 200 mg. stilboestrol daily. Greatly improved, able to walk
29.9.47	9.5	11	0	9.6	Liver not palpable
29.10.47	6.0	6.0	3.0	13	Condition still good

PROSTATE CASES (67) (continued)
Carcinoma of prostate with bone metastases (22) (continued)

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/100 ml.)	Notes
	Total	Alcohol-stable	Formal-dehyde-stable		
2	145	2.7	145	—	
3	117	3.7	113	—	
4	113	0.5	112	—	
5	50	5.2	47	42	
	21	—	17	81	
	2.5	—	2	18	
6	45	—	38	—	Stilboestrol treatment
	10	4.5	4.2	24	
7	30	1.3	28	—	
8	21	0.8	20	—	
9	20	3.4	18	—	
10	19	—	14	12	
11	16	7.3	8.7	32	
12	11	3.7	8.2	18	
	18	—	9	11	
13	18	1.8	15	—	
14	11	—	4.5	—	
15	9	0.5	9	—	
16	6.8	5.4	4.8	37	
17	4.6	4.6	1.2	—	
18	4.5	0.7	2.3	—	
	1.2	—	0.6	5.2	
19	3.8	3.8	1.5	—	
20	3.7	3.4	2.0	14	
21	3	2.1	0.5	—	
22	3	1.9	0.8	—	

Carcinoma of prostate; no bone metastases demonstrated (22)

Case	Total	Alcohol-stable	Formal-dehyde-stable	Alkaline	Notes
1	146	—	145	—	
2	14	3.0	12	15	
3	12	8.2	3.1	—	
	4.5	—	1.5	7.5	
4	11	8.8	2.5	—	
5	2.6	3.5	1.9	9	
6	9.5	—	3.7	14	
7	9	12	2.3	20	
	4.3	4.3	1.4	14	
8	7.9	—	6.8	—	Stilboestrol treatment
	3.9	—	1.5	—	
	2.5	—	1.4	—	
9	7.2	—	3.2	—	
10	6.8	5.4	4.8	16	
11	6.7	17	1.1	13	
12	6	0.7	0	—	Haemolysed
13	5.3	0.6	4.7	—	
14	4.7	—	1.3	—	
15	4.3	—	2.8	—	
16	4.2	3.3	3.0	—	
17	4.2	—	2.2	—	
18	4.0	4.5	1.8	—	
19	3.5	—	2.1	27	
20	3.0	2.0	3.0	—	
21	2.9	2.9	1.4	13	
	1.2	—	1.2	10	
22	2.9	—	0	—	

Other prostate cases (23)

Case	Total	Alcohol-stable	Formal-dehyde-stable	Alkaline	Notes
1	30	—	23	9	Senile hyperplasia
2	23	—	7.5	—	Retention
3	20	—	7.5	—	Adenoma
4	19	7.5	17	—	"
5	17	—	4.5	23	Retention
6	10	6.2	6.9	—	"
7	8.5	6.5	5.8	10	"
	6.0	—	4.0	—	
8	6.6	4.6	2.7	—	"
9	6.0	—	3.0	—	"
10	5.8	5.8	1.3	—	"
11	4.1	—	0	—	Adenoma
12	3.7	3.3	2.7	7.5	Senile hypertrophy
13	3.4	1.3	2.6	—	Retention
14	3.3	—	0	—	"
15	2.8	—	0.7	7.0	Adenoma
16	2.7	0.7	2.0	23	Senile hypertrophy
17	2.7	3.0	1.8	—	"
18	2.7	1.3	0.4	—	Adenoma "
19	2.4	—	0	—	"
20	1.9	1.9	0.7	—	Retention
21	1.8	2.0	1.8	—	"
22	1.8	1.5	0.7	—	"
23	1.7	0.4	1.7	—	Obstruction

CANCER OF VARIOUS ORGANS (24)

Case	Total	Alcohol-stable	Formal-dehyde-stable	Alkaline	Notes
1	13	—	1.6	15	Cancer of bladder
2	15	14	3.0	23	" " bone
3	9.5	—	4.0	25	" " "
4	3.6	3.0	2.7	47	" " breast with metastases
5	9.0	10	2.4	50	" " breast
6	2.2	3.6	2.2	—	" " bronchi
7	3.4	3.7	0	85	" " "
8	6.2	3.0	2.7	8.5	" " "
9	2.7	2.1	1.3	5.2	" " colon
10	2.3	1.8	0.4	9.0	" " "
11	13	—	1.6	15	" " lung
12	11	—	3.0	—	" " "
13	8.3	—	1.6	17	" " "
14	7.5	7.5	1.9	—	" " "
15	6.0	—	2.3	45	" " "
16	5.1	—	3.3	—	" " "
17	4.8	3.5	0.6	11	" " "
18	3.7	—	1.5	—	" " "
19	5.4	3.6	1.2	30	" " liver
20	4.1	4.0	1.8	33	" " "
21	5.2	4.5	3.7	—	" " pancreas
22	4.5	3.0	1.5	—	" " oesophagus
23	6.4	9.3	1.1	—	" " rectum
24	3.6	3.0	0	7.8	" " "

PAGET'S DISEASE OF BONE (15)

Case	Total	Alcohol-stable	Formal-dehyde-stable	Alkaline
1	12	7.6	3.3	20
2	7.5	8.0	5.5	140
3	6.7	6.0	0.4	15
4	6.7	5.5	3.4	15
5	6.3	5.4	2.9	75
6	5.2	5.2	1.1	81
7	4.5	3.0	1.5	76
8	3.4	3.0	0.4	9.1
9	3.1	2.9	0.8	30
10	4.1	—	0.5	62
11	4.0	—	1.3	—
12	2.9	2.2	1.4	20
13	2.7	—	0	—
14	2.3	2.5	0	25
15	2.3	—	0	—

LIVER DISEASE (38)

Case	Total	Alcohol-stable	Formal-dehyde-stable	Alkaline	Notes
1	9.6	—	1.2	17	Cholecystitis
2	2.5	2.0	0.2	—	"
3	3.7	0.8	1.5	14	Cirrhosis
4	3.0	3.0	2.7	13	"
5	2.2	0.9	0.4	22	"
6	2.4	3.6	1.8	7.8	"
7	18	15	3.7	—	Haematemesis, jaundice
8	7.8	6.8	3.8	150	Hepatitis
9	5.6	3.0	3.4	12	"
10	2.5	2.7	2.5	—	"
11	4.5	2.5	4.0	—	"
12	4.8	4.4	3.0	28	"
13	4.5	6.7	2.3	20	"
14	12	11	1.4	21	"
15	3.0	6.0	1.1	21	"
16	22	15	5.6	19	Hepatomegaly
17	13	15	4.8	82	Obstructive jaundice
18	8.8	6.2	5.6	63	" "
19	8.2	4.5	3.0	32	" "
20	7.5	3.8	3.8	21	" "
21	7.2	6.5	5.7	136	" "
22	6.3	6.3	4.9	37	" "
23	6.0	5.3	4.5	17	" "
24	6.0	3.6	1.8	40	" "
25	5.4	3.6	3.0	23	" "
26	5.2	3.8	4.5	31	" "
27	3.8	3.8	2.5	14	" "
28	3.3	2.3	1.8	21	" "
29	2.7	1.8	1.8	80	" "
30	2.6	1.2	0.7	20	" "
31	19	14	7.5	44	" "
32	6.3	3.8	1.8	90	" "
33	6.0	3.5	4.5	40	" "
34	2.7	1.8	1.8	80	" "
35	2.6	1.3	1.9	40	Ulcerative colitis
36	8.0	5.3	6.0	114	Xanthomatosis
37	7.6	5.6	4.9	105	"
38	3.2	3.2	3.0	122	"

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/100 ml.)	Notes
	Total	Alcohol-stable	Formal-dehyde-stable		
MISCELLANEOUS DISEASES					
1	6.0	—	3.0	—	Anaemia
2	1.5	1.5	1.5	—	"
3	6.0	4.5	3.0	—	Anaemia haemolytic
4	3.8	—	1.5	17	" "
5	3.6	7.8	2.4	6	" "
6	16	—	5.2	—	" and oedema
7	1.2	1.2	1.2	19	" pernicious
8	1.8	1.8	1.8	—	" "
9	5.0	3.8	1.4	—	" splenic
10	1.8	2.6	0.3	—	Arthritis
11	1.8	1.2	0.7	—	" "
12	1.6	—	0	—	" (rheumatic)
13	24	19	1.8	17	Bronchopneumonia
14	8.7	—	1.5	—	" "
15	6.2	—	1.8	—	Cystitis
16	2.3	2.3	1.4	11	Diabetes
17	2.0	0.5	0.5	—	" and uraemia
18	3.5	2.8	2.8	—	Dysurea
19	3.8	—	2.3	—	Epididymitis
20	4.8	4.2	2.0	5.5	Fracture
21	3.7	3.7	2.2	—	Gout
22	8.7	7.5	1.5	—	Heart failure
23	4.2	—	0	10	" "
24	2.2	2.0	0.7	—	" "
25	2.5	—	1.6	—	" and hypertension
26	12	—	6.0	—	Haemorrhage
27	2.2	1.5	1.8	—	" accidental
28	2.3	1.5	1.3	—	" "
29	17	19	4.5	—	Haematemesis
30	7.5	6.8	3.0	—	" "
31	4.7	3.3	2.7	—	Haematuria
32	2.4	1.8	0	34	Hodgkin's disease
33	5.9	8.5	3.1	12	Hypertension
34	2.2	—	0.7	20	Hypertensive heart disease
35	7.0	7.0	3.0	—	Hydronephrosis

Case	Acid phosphatase (units/100 ml.)			Alkaline phosphatase (K.A. units/100 ml.)	Notes
	Total	Alcohol-stable	Formal-dehyde-stable		
MISCELLANEOUS DISEASES (continued)					
36	4.8	4.2	3.0	17	Melaena
37	1.8	1.3	0	6.6	"
38	25	17	0	14	Myeloid leukaemia
39	4.5	4.5	2.2	—	Myxoedema
40	1.5	—	0.4	—	"
41	12	8.0	6.0	—	Nephritis
42	11	11	3.7	—	"
43	2.5	—	0.8	—	"
44	2.3	9.7	1.5	—	"
45	2.2	2.1	0.8	—	"
46	1.8	1.8	0.7	—	"
47	1.7	1.7	1.7	—	"
48	8.7	6.7	5.3	—	" chronic
49	5.3	3.8	1.5	—	" "
50	2.0	1.5	1.5	—	" "
51	1.8	4.2	1.8	9.6	" "
52	3.5	3.5	2.0	—	Nephrosis
53	6.0	6.7	1.8	—	Toxaemia of pregnancy
54	3.3	1.2	1.2	—	" " "
55	1.5	1.5	1.5	—	" " "
56	1.2	1.2	0.3	—	" " "
57	3.3	—	1.9	—	Papilloma of bladder
58	3.0	4.5	0	18	Peritonitis
59	16	—	5.2	—	Pyloric ulcer
60	12	—	6.6	48	Steatorrhoea
61	3.8	3.5	1.5	60	"
62	1.5	—	1.5	—	Stenosis, mitral
63	15	9.8	3.4	—	Stricture, urethral
64	2.6	1.8	1.8	—	Sprue
65	3.7	1.5	0.8	—	Hypertension
66	1.7	2.2	0.4	—	Thyrotoxicosis
67	1.3	—	0.4	—	Ulcerative colitis
68	3.8	3.8	1.1	10	Undiagnosed
69	2.7	6.0	2.0	—	"
70	4.5	—	0	—	"
71	2.7	—	0.7	11	"
72	6.0	3.0	1.5	—	Uraemia
73	2.1	1.4	1.0	17	Wilson's disease

A QUANTITATIVE PRECIPITATION TEST FOR SYPHILIS

BY

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In February, 1946, under the title of "A Preliminary Report on a Modification of the Kahn Test" (Price, 1946a), the author described a modification of the Kahn test, the advantages of which were stated to be: (1) that the results are more easily read than those of the Kahn test; (2) the results given by antigens prepared at different times and having different titres are identical; (3) the results obtained by this test appear to be somewhat more specific than those given by either the Kahn or Wassermann reactions. After more than 3,000 tests there seems to be no reason to modify these opinions.

One major disadvantage remained, that is, the difficulty in the preparation of the alcoholic extract of dried heart muscle according to Kahn's technique. If, moreover, one considers the production of the dried heart powder itself, the manufacture of Kahn antigen is outside the scope of most pathological laboratories. It is, therefore, desirable from all points of view to attempt to prepare an antigen which can be made in any laboratory, is specific in action, reasonably sensitive, and easy to handle.

Experimental

The starting point was the standard cholesterolized alcoholic extract of heart muscle used in the Harrison–Wyler (1929) technique of the Wassermann reaction. The lipoids were precipitated as described in the previous paper, and an attempt was made to work out a precipitation titre (Price, 1946b). Unfortunately, whilst positive sera gave fairly strong reactions all the negative serum and saline tubes contained a faint precipitate which was thought to be due to excess of cholesterol.

Accordingly, in the next experiment, Wassermann reaction alcoholic heart extracts containing varying quantities of cholesterol from 0.05 to 0.3 per cent were used. The precipitation titres obtained with the various antigens were:

W.R. alcoholic extract + 0.05% cholesterol 1 to 0.8 saline
  ,,   ,,   ,,   0.1%   ,,   1 to 1.0 saline
  ,,   ,,   ,,   0.2%   ,,   1 to 1.4 saline
  ,,   ,,   ,,   0.3%*   ,,   no titre obtainable; fine precipitate in all tubes.

*It should be pointed out that cholesterol at 0.3 per cent strength in the alcoholic extract required heat at 37° C. to effect solution and on regaining room temperature the cholesterol precipitated out from the solution.

When these antigens were tested by the method of optimal proportions (Price, 1946b), the best results were obtained when the ratio of serum to antigen was 5 to 1 and the precipitate of lipoids from 1 ml. of the heart extract (cholesterolized) obtained by using the precipitation titre was suspended in 0.6 ml. of saline. On the other hand, when used in a test, the less the alcoholic extract was cholesterolized the larger became the precipitates obtained with positive sera. Thus the best reaction was obtained with the alcoholic extract of heart muscle containing 0.05 per cent cholesterol.

Plain uncholesterolized alcoholic extract of ox heart was then used as an antigen in the manner already described for the cholesterolized extracts. The results of these experiments are summarized in Table I.

TABLE I

Alcoholic extract of ox heart 9 ml. to the gramme	Precipitation titre	Serum titre of reacting serum
Plain	0.8 ml. saline to 1 ml. heart extract	80 units
Plain + 0.05% cholesterol	0.8 ml. saline	80 units
Plain + 0.1% cholesterol	1.0 ml. saline	40 units
Plain + 0.2% cholesterol	1.4 ml. saline	20 units

It is evident that cholesterol as a sensitizer of this type of antigen was unimportant and could be omitted. The Wassermann reaction alcoholic extract of heart muscle used in the above experiments was obtained by extracting fresh wet ox heart muscle, cleaned from fat, with 9 ml. of absolute alcohol per gramme of muscle for 24 hours at 37° C. After filtration the alcoholic extract was placed in the ice chest (4° C.) for twenty-four hours and then refiltered.

Variations in the method of extraction were now investigated. Wet ox heart muscle was extracted for twenty-four hours, three days, and five days at 37° C. and at room temperature for three and five days respectively. All these extracts gave identical precipitation titres, and if cholesterol were added in the various amounts mentioned above (0.05 per cent, 0.1 per cent, 0.2 per cent) the precipitation titres varied accordingly. Furthermore, if serum quantitative tests on positive sera

were carried out using the same serum for each antigen, the unit values obtained varied in a similar manner to those obtained with the original extracts. In short, the method of extraction did not affect the precipitation reaction.

During these experiments it was noticed that when heat was employed in the extraction a deposit of crystals separated out from the alcoholic extract after about six weeks. These crystals could not be removed by filtration or centrifugalization, and they interfered with the reading of the tests as it was found that all tubes contained a fine deposit when examined with a ×6 lens. Up to the time that crystals started to appear there was no interference with the test and they were regarded as a non-specific by-product of the heat-induced alcoholic extraction. As a result of these experiments the use of heat in the alcoholic extraction of the heart muscle was abandoned and extraction at room temperature was adopted as the routine method.

Hitherto all extracts had been prepared by extracting one gramme of the ox heart muscle with 9 ml. of absolute alcohol. The next step was to ascertain what effect, if any, varying amounts of absolute alcohol per gramme of wet ox heart muscle would have on the quality of the antigen. The amounts used were 2.5 ml., 5 ml., and 15 ml. per gramme respectively. The results are shown in Table II.

The titrations given in the table below were repeated several times and the results obtained were always identical for the precipitation titres. At the same time unit values of sera of different strengths always bore the same proportion.

From these experiments it was concluded that

1. An alcoholic extract of ox heart obtained by using 2.5 ml. of absolute alcohol per gramme of muscle is useless as an antigen in this test.

2. The best results are obtained when an extract obtained by using 5 ml. of absolute alcohol per gramme of ox heart muscle is used.

3. The addition of cholesterol to the alcoholic extract of heart muscle in amounts of 0.05 per cent had no sensitizing action. Above this amount it inhibits the sensitivity of the extract as an antigen.

4. Whatever amount of absolute alcohol per gramme of ox heart is used to prepare an antigen, the best results are always obtained by using the plain extract without the addition of cholesterol.

It now remained to be shown which extract is the most sensitive antigen. Since plain alcoholic extracts

TABLE II

Each gramme of wet ox heart muscle extracted by	Antigen	Precipitation titre in terms of normal saline to each ml. of heart extract	Value of given serum (in units)
2.5 ml. of absolute alcohol ..	Plain extract	None obtainable: precipitate in all tubes	—
	Plain extract + 0.05% cholesterol	,, ,,	—
	Plain extract + 0.1% cholesterol	,, ,,	—
	Plain extract + 0.2% cholesterol	,, ,,	—
5 ml. of absolute alcohol	Plain extract	0.6 ml.	320
	Plain extract + 0.05 cholesterol	0.6 ml.	320
	Plain extract + 0.1% cholesterol	0.8 ml.	80
	Plain extract + 0.2% cholesterol	1.0 ml.	40
15 ml. of absolute alcohol ..	Plain extract	0.8 ml.	20*
	Plain extract + 0.05% cholesterol	1.0 ml.	20
	Plain extract + 0.1% cholesterol	1.2 ml.	20
	Plain extract + 0.2% cholesterol	1.6 ml.	20

*Whilst this titre could not be read at higher than 20 units, the precipitate was somewhat heavier than those given by the other antigens in the same class.

of ox heart muscle had always proved the most sensitive of their group (with or without cholesterol added), the three plain extracts obtained by using 5 ml., 9 ml., and 15 ml. of absolute alcohol respectively per gramme of heart muscle were used as antigens in parallel with different sera of varying strengths. Many hundreds of sera were tested, but Table III gives a sample of ten sera tested at the same time.

The ten sera shown in the table were unselected except in so far as they came from one batch of sera tested by the Wassermann reaction. Of the antigens employed, it will be seen that that obtained by extracting 1 g. of ox heart muscle with 5 ml. of absolute alcohol is the most sensitive, whilst between the other two there is little to choose. These results were repeated many times with similar results.

To conclude the experimental work the sensitivity of the antigen as described in the first paper was compared (Price, 1946c) with the sensitivity of the antigen prepared in the above manner. More than 1,000 sera were tested in parallel, and it was found that sera yielding 0 units with the one antigen always gave a similar result with the other. On the other hand, when sera yielding positive results were encountered, considerably higher values were given by the latter antigen than by the former. This may perhaps be best shown in the form of a graph (Fig. 1).
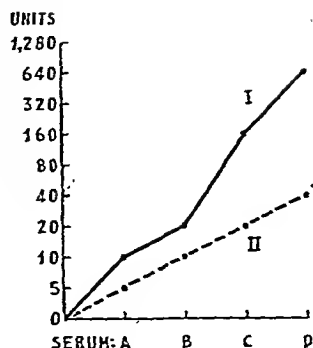
FIG. 1.—(I) Plain alcoholic heart extract antigen used. (II) Antigen prepared from Kahn extract.

The Test Proper

The above experimental work is a continuation of that contained in the first paper (Price, 1946a), and the test about to be described, henceforth referred to as the P.P.R.* for syphilis, is based on the technique as set out therein.

Apparatus required.—The necessary apparatus is as follows: Wassermann tubes 5 cm. × 1.25 cm.; Wassermann racks to hold tubes; pipette† to hold 5 volumes (0.11 ml.); droppers† to hold 5 volumes (0.11 ml.), for saline used in conjunction with Donald's dropping apparatus; 1 volume (0.022 ml.); shaking apparatus, 280 oscillations per minute.

*Price's Precipitation Reaction.
†The above volume (0.022 ml.) is purely arbitrary and was adopted since the necessary pipette and droppers were already employed in Harrison Wyler technique of the Wassermann reaction (M.R.C. report 1929) used in this laboratory. In addition it ensured a reasonable economy of serum and antigen.

TABLE III

Serum No.	Antigen prepared by extracting 1 g. of ox heart with 5 ml. of absolute alcohol	Antigen prepared by extracting 1 g. of ox heart with 9 ml. of absolute alcohol	Antigen prepared by extracting 1 g. of ox heart muscle with 15 ml. absolute alcohol	W.R. result	Kahn test – result
1	40 units	10 units	10 units	+ +	Positive
2	80 units	10 units	10 units	+ +	Positive
3	320 units	320 units	320 units	+ +	Positive
4	40 units	5 units	5 units	+ +	Positive
5	0 units	0 units	0 units	Neg.	Negative
6	10 units	5 units	5 units	+ +	Positive
7	320 units	160 units	80 units	+ +	Positive
8	320 units	40 units	40 units	+ +	Positive
9	160 units	40 units	40 units	+ +	Positive
10	40 units	5 units	5 units	+ +	Positive

Preparation of the alcoholic heart extract.—An ox heart obtained from a freshly killed beast is obtained, stripped of fat, cut into small pieces, and weighed. The muscle is ground in a mortar for one minute with a few ml. of the alcohol and glass powder. The mixture is then transferred to a suitable glass-stoppered bottle and the mortar washed out with a further few ml. of the alcohol, the washings being then poured into the bottle. Sufficient alcohol is now added so that the bottle contains 5 ml. of alcohol for every gramme of heart muscle. The bottle is well shaken for a few seconds and placed in a dark cupboard or box for three days at room temperature, being shaken two or three times each day. At the end of this time the contents of the bottle are filtered into another glass-stoppered bottle, which is then placed in the ice chest (4° C.) for 24 hours. Finally, whilst still cold, it is refiltered into a bottle of brown-coloured glass (to exclude light). The very pale-lemon-coloured filtrate is then ready for use.

Precipitation titre.—Before the antigen can be prepared for use in the test proper, the precipitation titre must be determined. This is done as follows. Four mixtures of 1 ml. of the alcoholic heart extract are made with respectively 0.4 ml., 0.6 ml., 0.8 ml., and 1 ml. of normal saline. The mixtures should be made by gently pouring the alcoholic extract down the inside of the small tube containing the saline and then pouring back into the tube which originally contained the extract. Gentle but thorough mixing is obtained by pouring and repouring seven times in all. The mixtures are allowed to stand for 30 minutes. The precipitate which forms in each tube is packed tightly into the bottom of the tube by centrifugalization (2,000 r.p.m.) for ten minutes, at the end of which time the supernatant fluid can be removed without fear of loss of any of the precipitate. The tubes are allowed to drain for five minutes by standing in the inverted position. Normal saline (0.6 ml.) is added to each precipitate, and thorough mixture obtained by means of a Wright's capillary pipette. Thus four antigens are obtained, and these are titrated by adding one volume of each antigen to five volumes of known moderately positive serum, one volume to five volumes of normal serum, and one volume to five volumes of saline. After five minutes on a shaking machine, 1 ml. of saline is added to each tube. The rack containing the tubes is gently agitated and the results read by means of a ×6 lens and a slit lamp. The precipitation titre is indicated by the tubes showing no

precipitate and containing the antigen obtained by adding the least amount of saline to 1 ml. of the alcoholic heart extract. These tubes containing no precipitate are of course always in the normal serum and saline rows. The tubes containing moderately positive serum are not essential for the titration but are included for the sake of completeness.

This titration may be summarized as in Table IV. From the Table it is clear that the least amount of saline to be added to 1 ml. of the alcoholic extract (5 ml. to the gramme) of ox heart muscle in order to obtain a suitable antigen is 0.6 ml., and this constitutes the precipitation titre. It is a matter of experience that, although very many of these extracts have been titrated, the precipitation titre has always been 0.6 ml. of normal saline to 1 ml. of the alcoholic extract of ox heart muscle. Furthermore, once this titre is determined for any given extract it remains constant. The amount of normal saline (0.6 ml.) added to the precipitate to form the antigen has already been determined by the method of optimal proportions (Price, 1946b).

Preparation of antigen for use in the test proper.—Having ascertained the precipitation titre as 0.6 ml. of normal saline to 1 ml. of heart extract, a simple proportion sum will give the amounts of each reagent to mix to produce the amount of antigen necessary for a given number of tests. The practice in this laboratory is to make up enough for at least a week's work. To make 12 ml. of the antigen (enough for 300 to 400 serum tests) add 20 ml. of the alcoholic heart extract to 12 ml. of normal saline. This operation is performed by measuring 20 ml. of the alcoholic extract in one 50 ml. centrifuge tube and 12 ml. of normal saline into a similar tube. The alcoholic extract is first poured on to the saline, and then the mixture is poured back and forth six times, subsequently being allowed to stand for 30 minutes. (It is of the utmost importance that the *alcoholic extract be poured on to the saline* in the first instance, otherwise a very fine non-specific precipitate is apt to appear in all tubes of the test. This may confuse the readings,

TABLE IV

Alcoholic extract of ox heart 5 ml. to 1 g.	Normal saline added to obtain precipitate	Saline added to precipitate to form antigen	Reaction precipitate		
			Syphilitic serum	Normal serum	Saline
1 ml.	0.4 ml.	0.6 ml.	+	+	± Nil
1 ml.	0.6 ml.	0.6 ml.	+ +	Nil	Nil
1 ml.	0.8 ml.	0 6 ml.	+ +	Nil	Nil
1 ml.	1.0 ml.	0 6 ml.	+ ±	Nil	Nil

especially at the end-points of quantitative tests.) At the end of this time the precipitate which has _formed is swung to the bottom of the tube by centrifugalization (2,000 r.p.m.) for 10 minutes. The supernatant fluid can then be poured off with safety and any remaining fluid allowed to drain out by placing the tube for five minutes on a filter paper in the inverted position. Any fluid still left on the sides of the tube can be wiped off with a towel and 12 ml. of normal saline added to the precipitate at the bottom of the tube. The mixing of the precipitate and the saline is accomplished by means of a Wright's capillary pipette, and the resulting suspension is transferred to a brown glass bottle either with a glass stopper or vulcanite screw cap. Tinfoil stoppers are to be avoided, since the antigen is acid enough to set up a reaction with the metal. In order to avoid infection of the made-up antigen, 0.022 ml. of sodium azide, 6.8 per cent, is added to each ml. of the suspension. When not in use the antigen should be kept in the ice chest at 4° C. In addition the antigen pipette should have a cotton-wool plug at the teat end and should be rinsed out with acetone and dried before use. If kept thus, the antigen remains active for months.

Sera.—The sera to be tested are obtained in exactly the same manner as those subjected to a Wassermann reaction and are inactivated for thirty minutes at 56° C.

SCREEN TEST

Each serum to be tested has two tubes allotted to it. Into one is put 5 volumes of neat serum and into the other 5 volumes of serum diluted 1 in 2 with saline. When testing large numbers of sera it is advisable to use the saline dropper in conjunction with Donald's dropping apparatus. This will deliver automatically five volumes of saline per drop, and this five volumes of saline can be rapidly delivered into each tube of the back row. Five volumes of the appropriate neat serum can then be added to each of the respective tubes of the front and back rows, five volumes from the latter being discarded after thorough mixing.* One volume of antigen is now added to all tubes necessary for the batch of sera under test.

Screen Test

Tube 2	5 volumes of serum diluted 1 in 2
	1 volume of antigen

---

*It had been hoped to make the screen reaction a one-tube test using neat serum alone. With experience it was found that some strongly reacting sera contained too much reagin for the amount of antigen employed, thus suppressing the precipitation reaction. This zone phenomenon can be overcome successfully by using the two-tube technique.

Tube 1	5 volumes of neat serum.
	1 volume of antigen.

The rack containing the tubes is placed for five minutes in a shaking apparatus (280 oscillations per minute). Subsequently 1 ml. of normal saline is added to each tube. The contents of the tubes are now well mixed by gentle agitation of the rack, and the results read by means of ×6 lens and the slit lamp. When reading results the tube should be held on the slant and the particles, if any, looked for in the elliptical disc formed by the top of the fluid. The lowest positive reading is recorded if small discrete greyish particles of similar size are seen. Stronger reactions cause a greater number of particles which agglutinate and form a greyish-white precipitate, whilst the strongest reactions result in particles easily visible to the naked eye. These particles appear as large white flocculi. In tubes in which no reaction takes place the fluid contains no particles and is of a uniform clarity.

The sera giving negative results are recorded, and those giving positive results are dealt with by means of the quantitative test.

QUANTITATIVE TEST

The quantitative test is set up on the principle of diluting the serum by a series of twofold dilutions.

Four tubes are allotted to sera giving weak reactions, and eight tubes to those giving strong reactions. All tubes except the first receive five volumes of saline, and if large numbers of positive sera are to be dealt with it is advisable to use the saline dropper in conjunction with Donald's dropping apparatus. The first and second tubes each receive five volumes of the serum to be tested. The contents of tube 2 are well mixed, and five volumes are carried over to tube 3. The mixing and carrying is repeated from tube to tube in serial order until the last one is reached, when five volumes of the mixture are discarded leaving five volumes of this dilution in the tube. Thus the series of tubes allotted to each serum contains one of neat serum, and dilutions from 1 in 2 to 1 in 128 depending on the number of tubes used. All tubes now receive one volume of the antigen and are shaken for five minutes on the shaker.

Strongly Reacting Serum		*Quantitative Test*	
5 volumes of serum diluted			
Tube 4	1–8	Tube 5	1–16
Tube 3	1–4	Tube 6	1–32
Tube 2	1–2	Tube 7	1–64
Tube 1	Neat serum	Tube 8	1–128

With high titre serums the dilutions may have to be carried further.

One ml. of saline is then added to each tube by means of the simple apparatus shown in Fig. 2.
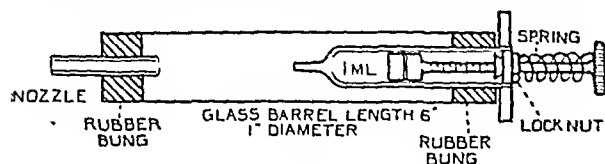
Fig. 2

The barrel of the apparatus holds approximately 30 ml. of saline, and each time the plunger is pushed home against the spring 1 ml. is delivered through the nozzle when once the locknut on the plunger inside the small syringe has been suitably adjusted. This simple apparatus saves much time and labour. Mixing of the contents is ensured by gentle agitation before reading with ×6 lens and slit lamp. The tube containing the highest dilution of the serum which gives the lowest positive reading (that is, small discrete greyish particles of similar size seen in the elliptical disc at the top of the slanted tube) is noted. From this the number of units is calculated. For example, if the tube containing neat serum is the only one to show any particles (this is the lowest positive reading) the titration is recorded as 5 units on the grounds that no serum test is proven to be sensitive enough to demonstrate the extreme limits of the small amounts of reagin present in some syphilitic sera. If, on the other hand, the highest dilution of serum recording a positive reaction is 1 in 64, then the titre of the serum is recorded as 320 units. Occasionally sera from patients suffering from florid secondary syphilis or multiple gummata record positive readings above the eight-tube dilution, the record so far being the tenth tube (2,560 units).

Thus the readings of the test are recorded in terms of units such as 0, 5, or multiples of five, and are designed to give a rough idea of the relative activity of the disease.

Cerebrospinal Fluid

This test has been adapted to testing the cerebrospinal fluid, and, although the numbers tested as yet are relatively small, no fluid has been met which recorded any degree of positivity with the Wassermann reaction and failed to record a positive P.P.R. for syphilis.

The technique is similar to that used with the serum, and is as follows:

By the principle of optimum proportions it has been ascertained that the best results are obtained when 15 volumes of cerebrospinal fluid are used with 1 of antigen.

Screen test.—Each cerebrospinal fluid has one tube allotted to it, into which one volume of antigen and 15 volumes of cerebrospinal fluid are placed and shaken for five minutes. At the end of this time 0.5 ml. of saline is added to each tube, the contents of which are mixed by gentle agitation and the results read in the manner described for sera. All positively reacting fluids are put up for a quantitative test.

Quantitative test.—Each cerebrospinal fluid is allotted eight tubes. All tubes excepting the first receive fifteen volumes of saline, and the first and second tubes receive fifteen volumes of cerebrospinal fluid each. Serial dilutions of from 1–2 to 1–128 are made by mixing and carrying over fifteen volumes from tube to tube until the last is reached, when, after mixing, fifteen volumes of the mixture are discarded. Thus each positive cerebrospinal fluid has a series of tubes containing fifteen volumes of the neat fluid and fifteen volumes of a series of dilutions from 1–2 to 1–128. Each tube now receives one volume of the antigen and is shaken for five minutes, and subsequently 0.5 ml. of saline is added. Mixing of the contents of the tube is secured by gentle agitation and results read and recorded in the same manner as for sera.

Cerebrospinal fluids require no treatment and are suitable for test provided they are reasonably free from foreign matter. The highest recording from a cerebrospinal fluid to date is 160 units.

Results

Over 8,000 routine tests have been done in parallel with the Wassermann reaction and Kahn test, with extremely satisfactory results. In order to assess the value of this test as against the Wassermann and Kahn tests, the results of 2,936 routine parallel tests have been examined. These tests were the yield of three months' routine testing and are unselected. The results were as follows:

Agreements

Negative reactions	..	2,459 (83.7%)
Positive reactions	..	358 (12.2%)
Total	..	2,817 (95.9%)

Disagreements 119 (4.1%)

Of these the following analysis may be made:
Group 1. P.P.R.   negative
          Wassermann reaction negative
          Kahn reaction   positive 66 sera.

Group 2. P.P.R.   positive
     Wassermann reaction negative
     Kahn reaction   positive   50 sera.

Group 3. P.P.R.   positive
     Wassermann reaction negative
     Kahn reaction   negative   1 serum.

Group 4. P.P.R.   negative
     Wassermann reaction positive
     Kahn reaction   negative   1 serum.

Group 5. P.P.R.   positive
     Wassermann reaction positive
     Kahn reaction   negative   1 serum.

Examination of the case cards of those patients whose sera gave conflicting results reveals that they were grouped into the following six types of case:

1. *Primary syphilis untreated.*—In this group there were 3 sera, all of which recorded positive Kahn tests. One serum reacted positively with the P.P.R. and negatively with the Wassermann reaction, whilst two sera gave negative results with these two tests.

2. *Latent syphilis treated.*—This was the largest class, consisting of 95 sera. All the patients had been treated with penicillin followed by arsenic and bismuth. All the sera recorded negative Wassermann reactions. There were 3 results which were possible false positive Kahn reactions. For the rest, the noticeable feature was that usually the Wassermann became negative first, then the P.P.R., which was followed a month or two later by the Kahn test. A reversal of a negative P.P.R. to positive occurred on only two occasions.

3. *Latent syphilis untreated.*—In this group there were only two sera, both of which recorded negative Wassermann reactions, but positive P.P.R.s and Kahn tests.

4. *Old cured syphilis.*—One serum was tested from a patient who had had syphilis in 1924. He came up for a check blood test, but showed no signs or symptoms. In a series of three tests done within a month the Kahn was positive on one occasion, whilst the Wassermann and P.P.R. were negative at each testing.

5. *Patients attending for treatment for gonorrhoea.*—There were 12 of these sera, 9 of which recorded negative Wassermann and P.P.R. reactions, but positive Kahns. Of these 9 patients, who were under observation for months, none revealed any clinical or serological evidence of syphilis, apart from the Kahn test. The remaining 3 were problem sera. One gave persistently positive Kahn and P.P.R. results with negative Wassermann reactions over a period of three months. He was given 150,000 units of penicillin at the outset of his gonorrhoeal infection, but after two months there was no evidence of syphilis. He remains under observation. Patient 2 was a coloured patient who reported that he had had yaws as a youth. He attended with gonorrhoea and received 150,000 units of penicillin, followed by a further 200,000 units. Over a period of three months he persistently recorded negative Wassermann and positive Kahn and P.P.R. reactions. There was no clinical evidence of syphilis, and he remains under observation. Patient 3 attended with gonorrhoea, recording a negative Wassermann but a positive Kahn and P.P.R. There was no evidence of syphilis and he was given 150,000 units of penicillin. Six days later his blood recorded all three tests as positive. Unfortunately the patient defaulted so no opinion could be given, but possibly he had a double infection of gonorrhoea and syphilis, the latter being first detected by the P.P.R. and Kahn tests.

6. *Sera from patients in whom no evidence of venereal disease was found.*—There were six of these sera, all of which recorded negative P.P.R. and Wassermann reactions but positive Kahn tests. None of these patients whilst under observation revealed any evidence of syphilis in spite of repeated clinical examination and blood tests.

PRIMARY SYPHILIS

In order to ascertain how soon after infection the P.P.R. becomes positive, the case records of all patients attending the Whitechapel Clinic and suffering from untreated primary syphilis during a period of six months were examined. The number totalled 80. *Spirochaeta pallida* had been demonstrated in the primary lesions in all cases.

Although it is difficult to be certain of the exact date of the infecting exposure, it was thought to be of sufficient interest to attempt to ascertain how soon after an exposure a positive P.P.R. would be recorded. Of 38 patients in the first four weeks after exposure, the serum of 19 (50 per cent) recorded a positive P.P.R. Of 29 patients in the fifth to eighth week after exposure the sera of 16 (55 per cent) recorded positive P.P.R. reactions. Finally, of 13 patients in the ninth to twelfth weeks after exposure the sera of 7 (53 per cent) recorded positive P.P.R. reactions. It should be noted that the parallel Wassermann reactions and Kahn tests were identical.

CEREBROSPINAL FLUID

During this investigation a total of 284 cerebrospinal fluids were tested in parallel with the Wassermann reaction. Of these, both tests recorded 13

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positive and 271 negative reactions. The highest unit value yet recorded by the test with cerebrospinal fluid was 160, and it appears that values of 5 to 10 units suggest a condition of tabes dorsalis, whilst higher values are more typical of fluids obtained from patients suffering from general paralysis of the insane.

Comment

Whilst this examination of results is not regarded as exhaustive, it does seem to show that the P.P.R. is at least as sensitive as the Wassermann reaction and somewhat more specific than the Kahn test. Thus, of 9 patients suffering from gonorrhoea and 6 in whom no signs of venereal disease were discovered, all recorded negative P.P.R. but positive Kahn tests. These latter seem to be false positives, particularly as the Wassermann as well as the P.P.R. reactions were negative. They represent 15 (12 per cent) out of the total of 119 conflicting results recorded by parallel Wassermann, Kahn, and P.P.R. reactions respectively, or 0.5 per cent of the total number of sera (2,936) examined. Of these same sera only 2 appeared to give false positive P.P.R. reactions.

Consideration of the results obtained by this test suggests that the quantitative test gives some indication of the activity of the disease. In general terms it may be said that the greater the number of units recorded by a serum, the more active is the spirochaetal infection. Sera from patients suffering from florid secondary syphilis or multiple gummata always record a high unit value, and the cerebrospinal fluids from patients suffering from general paralysis of the insane record higher readings than the cerebrospinal fluids of tabetics.

Summary

1. The experimental work underlying a serum precipitation test for syphilis is given.

2. The preparation of the alcoholic extract from the raw ox heart is described. This operation is well within the capacity of any moderately well-equipped laboratory.

3. The preparation of the antigen from the alcoholic extract of ox heart and its standardization for use in the test is given in detail.

4. The screen test as described is simple, speedy, reliable, and easily read.

5. The method for the quantitative test is given; the test gives some idea of the activity of the disease.

6. The testing of cerebrospinal fluids (which require no prior preparation) is shown to give results equal to those of the Wassermann reaction.

7. The results of 2,936 routine parallel tests with the Wassermann and Kahn tests are examined.

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CHLORAL HYDRATE PLATES FOR THE INHIBITION OF SWARMING OF PROTEUS

BY

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In routine bacteriology the swarming of *Proteus* on plate cultures often masks the identity or presence of other bacteria which may be present. Mixed cultures including *Proteus* are often obtained from open wounds or sinuses following abdominal or chest operations, so that unless some special selective medium is used it is usually impossible to identify or to isolate other bacteria from a culture containing *Proteus*. Frequently β-haemolytic streptococci are missed in such cultures, and thus one often fails to identify an organism causing some infection which may have become secondarily infected with *Proteus*.

Numerous methods have been published for the inhibition of swarming of *Proteus* on culture plates, the simplest being the use of 6 per cent agar (Hayward and Miles, 1943). This high concentration of agar often leads to technical difficulties in preparation, and if the plates are not sufficiently dried, or if a very moist specimen is inoculated, *Proteus* is not necessarily prevented from swarming. Many chemicals have been incorporated in agar to make selective media, the most popular method being that of adding 1/5,000 sodium azide (Snyder and Lichstein, 1940). Unfortunately, when sodium azide blood-agar plates are used the blood tends to haemolyse and the plates do not keep satisfactorily before use. Jones and Handley (1945) described a satisfactory highly selective medium for the isolation of *Salmonellae* from material contaminated with *Proteus*.

In 1931 Krämer and Koch reported the results of their experiments using 14 different types of media for the inhibition of swarming, and showed how the addition of chloral hydrate prevented the swarming of *Proteus*. In this country the practical use of chloral hydrate plates appears to be little known except in certain laboratories ; this paper has therefore been written in order to bring it to the knowledge of everyone as being a practical method.

After certain preliminary experiments it has been found that chloral hydrate in a final concentration of 1/1,000 in nutrient agar or blood agar inhibits the swarming of *Proteus* but allows good growth of *Proteus* and of most other bacteria. Experience with the method coincides with that of Krämer and Koch, who found that practically all bacteria produced typical colonies. One great advantage is that it allows good growth of β-haemolytic streptococci, and also permits the production of β haemolysis, which thus enables one to detect and isolate single colonies from cultures heavily infected with *Proteus*. It is an advantage to incubate the plates for a full 24 hours, although after 18 hours the β haemolysis of haemolytic streptococci can usually be detected with ease.

On 1/1,000 chloral hydrate plates colonies of *Proteus* appear as discrete colonies from 1 to 2 mm. in diameter, so that if necessary one can pick off single colonies of *Proteus* and also single pure colonies of other bacteria from mixed cultures. This is an advantage over media which inhibit the growth of *Proteus*, as on them colonies of other bacteria need not necessarily be pure, as the *Proteus* bacilli just fail to grow but lie dormant and live on the plate. Weaker concentrations, such as 1/1,500, allow the colonies of *Proteus* to become large and semi-confluent, making it difficult to isolate other types of colonies present. At 1/2,000 slight spreading occurs.

Stronger concentrations inhibit the growths of *Proteus* and of other bacteria, but also inhibit the production of β haemolysis on blood-agar plates by haemolytic streptococci.

Preparation of Plates

The method of preparation of chloral hydrate plates is simple. A stock 10 per cent solution of chloral hydrate is made in distilled water and an appropriate amount added to the medium to give a final strength of 1/1,000. It is an advantage to make several plates at one time, adding the chloral hydrate to the bulk base at the time of pouring the plates, otherwise a very small volume must be added to the medium for each plate. It is not necessary to sterilize the stock solution, as it is apparently self-sterilizing at this strength, but it is an obvious advantage to exclude bacteria, especially spore-bearers, by using sterile distilled water. If preferred, the stock solution may be autoclaved without interfering with its inhibitory properties. Once the plates are poured they should be dried in the usual way, but not overdried.

The chloral hydrate blood-agar plates may be stored in the refrigerator for a week before use without the occurrence of haemolysis. Any stored plate should be dried in the incubator for about half an hour before inoculation.

Krämer and Koch report having found occasional batches of blood-agar plates giving atypical growths, but over the last three years no very unusual growth has been found as long as the correct percentage is accurately added to the media. Slight increase in the concentration due to the inaccurate addition of too small a volume of stock solution will lead to irregular results.

Conclusions

1. Chloral hydrate in a final concentration of 1/1,000 in nutrient agar or blood-agar plates inhibits the swarming of *Proteus*.

2. The chloral hydrate in the strength recommended permits the growth of *Proteus* as single colonies 1 to 2 mm. in diameter.

3. Chloral hydrate in this strength allows the production of β haemolysis on blood-agar plates by haemolytic streptococci.

4. Chloral hydrate plates permit the identification and isolation in pure culture of bacteria from mixed cultures contaminated with *Proteus*.

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REVIEWS

Illuminants and Illumination for Microscopical Work. Monograph III of the Quekett Microscopical Club. By F. E. Ockenden. London. Williams and Norgate: for the Quekett Microscopical Club, 1947. Pp. 26. Price 2s. 6d. net.

Most microscopists know little of the principles which determine the choice of illuminants and illumination for microscopical work. The third of the Quekett Microscopical Club's monographs should, therefore, be of considerable interest to all those who use the microscope and are anxious to obtain the best critical illumination possible.

Before light sources are discussed, various terms and definitions are given, such as "Candle" (symbol I), "the standard of luminous intensity," "Flux" (symbol F) and "foot candle" (f.c.), a special case of the more general unit of illumination, and "lumens per unit" (symbol E). The reader is then introduced to the ideas and importance of directions and "mean spherical" candle-power and colour temperature. Closely related to the subject of colour temperature is the spectral sensitivity of the human eye. The high sensitivity of the eye and also of the selenium (barrier layer) photoelectric cell for green, combined with the large proportion of this radiation normally available, is the reason why a wavelength of 0.55 μ is so popular. The green filter is not only very comfortable for long periods but the relatively short wavelength transmitted helps to procure the maximum possible resolution for an objective of given aperture. The advantages of photoelectric cells both of the vacuum and selenium or barrier layer pattern are fully discussed. The chief disadvantage is that the response to both the blue and the red is relatively too high. An extensive survey of illuminants, including daylight, is provided and many important tips are given such as the fact that the life of tungsten filament lamps may be prolonged if one or two low-voltage taps in the transformer are available. Thus a transformer nominally rated at 6 V may have additional terminals rated at 5, 4, and even 3 V. The life of a lamp when connected to the 5-V tapping is increased two to threefold and that on the 4-V tapping is almost unlimited. Projection lamps are an important type of illuminant: they range from the simple motor-car headlight, rated at some 24 W, to the multiple filament pattern with ratings of 50 to 500 W. In the ribbon filament type the spiral construction is avoided. Finally the illuminating system is discussed and consideration is given to the arrangement of the microscope substage and ancillary apparatus with low, medium, and high power, and high intensity illumination—the Köhler system.

This monograph is so full of important data that it might well find a place among those few books which the pathologist must keep within easy reach of his working bench.

G. M. FINDLAY.

Recent Advances in Pathology. By Geoffrey Hadfield and Lawrence P. Garrod. London. J. and A. Churchill. Fifth edition, 1947. Pp. 361. 60 illustrations. Price 21s. net.

Much new material has been fitted into the present edition without disturbing the plan or size of this well-known book in the "Recent Advances" series. Illustrations, mostly excellent photographs, are included where most needed. But in a subsequent edition a plate more in keeping with the others should be found to illustrate arsenical dermatitis and cancer (Fig. 9).

The chapter on nephritis, based as it is on the published work of Ellis, is greatly improved, and it is hoped that present concepts on renal damage may prove to be as near the truth as it is possible to get. Liver disease in the light of present knowledge is carefully reviewed, but the term "homologous serum hepatitis," though in common use, surely deserves a more appropriate synonym.

Much new work has been done on the relationship of enzymes and hormones to the cancer problem. Most of the available data has been carefully sifted and edited. The conclusion is drawn that malignant disease is due to intracellular disturbance, in nature chemical or even "microbic."

The chapter on ductless glands, contributed by Dr. E. F. Scowen, is a clear exposition of the facts. The author is at pains, and rightly, to differentiate between (1) hormones proper and (2) hormonally active substances recovered from the urine even though chemical constitution of the latter suggests a specific organ origin. It is not true to state that any given hormone is the prerogative of any one gland.

The subject index is carefully arranged and the name index should be a most valuable aid to bibliography.

MAGNUS HAINES.

We regret to announce the death of Dr. E. ff. Creed, Director of Pathology at King's College Hospital, London. An obituary notice will appear in the May issue.

ABSTRACTS

This section of the Journal *is published in collaboration with the two abstracting journals,
Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published
by the British Medical Association. In this* Journal *some of the more important articles on subjects
of interest to clinical pathologists are selected for abstract, and these are classified into four sections:
bacteriology ; biochemistry ; haematology ; and morbid anatomy and histology.*

BACTERIOLOGY

**Isolation of Poliomyelitis Virus from the Throats of
Symptomless Children.** Howe, H. A., and Bodian, D.
(1947). *Amer. J. Hyg.*, 45, 219.

Poliomyelitis virus was isolated from 1 of 3 patients
suffering from the disease and from 1 of 6 juvenile con-
tacts but from none of 5 adult familial or 7 juvenile and
6 adult extrafamilial contacts of these cases. Virus was
found in the throats of 2 out of 28 healthy children
frequenting a neighbouring playground. One year later
these children had developed antibodies to the virus
isolated from them.   *J. B. Ellison.*

**An Outbreak of Diphtheria in a Highly Immunized Com-
munity.** Fanning, J. (1947). *Brit. med. J.*, 1, 371.

A small outbreak of diphtheria developed in a girls'
school where 94% of the children had been immunized.
Eight pupils developed diphtheria during the half-term
holiday, but swabbing showed no carriers in the school
itself. Two clinical cases later developed in non-
immunized children, and after Schick-testing (read at
24 hours) 53% were recorded as Schick-positive and
passively immunized by injecting 0.3 ml. of A.P.T. and
500 units of antitoxin. In spite of this 4 more cases
developed, and reswabbing showed 8 carriers of *C.
diphtheriae*. Further cases occurred during the next
holiday, and 3 months after the onset of the epidemic
swabbing of the school gave negative results. A further
dose of A.P.T. was then given to all children. It is
recommended that boosting doses of diphtheria prophy-
lactic be given every 3 years during school life to
maintain immunity.

**The Agglutinative Reaction for Hemophilis Pertussis
Following Whooping Cough and Following Immuniza-
tion.** DeGara, P. F., and Mayer, S. A. (1947). *J.
Pediat.*, 30, 171.

In this investigation of agglutination reactions for
Haemophilus pertussis, serum was collected from 130
children aged 1 to 12 years and comprising 4 groups:
(1) children with a history of pertussis 3 months to 11
years previously; (2) children who had had a prophy-
lactic course of pertussis vaccine 6 weeks to 9 years
before; (3) children who had had a course of pertussis

antigen, "tri-immunol"; (4) controls with no history
of pertussis or prophylaxis. The results of the agglutina-
tion reactions in each group are shown in the diagram.
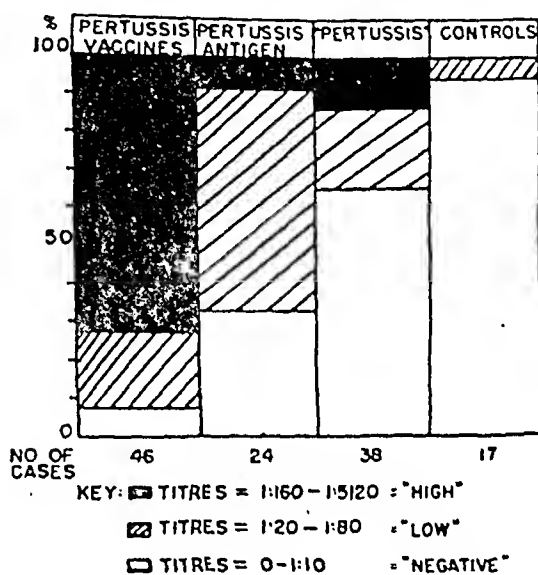
It is concluded that agglutination reactions are of
limited value in determining the state of immunity of a
child who is alleged to have had pertussis. Prophy-
lactic vaccination is followed by a high agglutinin titre
in a majority of cases.   *M. MacGregor.*

Typhoid Fever in Vaccinated Laboratory Workers.
Headicke, T. A. (1947). *J. infect. Dis.*, 80, 113.

A brief account is given of four cases of mild and
atypical typhoid fever in vaccinated laboratory workers
engaged in typhoid research and the preparation of
typhoid vaccine.
Diagnosis in each case was confirmed by positive blood
culture. The agglutination results in two cases showed
H-titres rising from zero to 320 and O-titres rising from
zero to above 1,000. The inoculation history indicated that
both patients had received full immunizing courses of

vaccine with booster doses within 6 months of the onset of the disease. After reviewing some recent literature on typhoid in inoculated individuals the author concludes that typhoid vaccine, where it does not prevent infection, so modifies the condition that the clinical manifestations are atypical and the course of the disease is mild except in massive infections.　　　　　　　　　*H. J. Bensted.*

Human Glanders : Report of Six Cases. Howe, C., and Miller, W. R. (1947). *Ann. intern. Med.*, 26, 93.

Six cases of laboratory workers engaged in research with *Malleomyces mallie* are reported. The incubation period was estimated at between 10 and 14 days, and the route of infection was probably the respiratory tract. Organisms were not recovered in spite of numerous attempts, but agglutination titres rose above 1 in 320 in 5 patients, and all 5 developed positive skin tests. The sixth case showed a rise of agglutination titre to 1 in 320 on one occasion only, the other tests being negative. Chemotherapy with sulphadiazine gave inconclusive results.

Ulcerative Colitis due to Fusospirochaetal Infection. (Colitis ulcerosa Fuso-spirochaetosa. Bericht über eine infektiöse Form der colitis ulcerosa im deutschen Konzentrationslager Sutthof b. Danzig in den Jahren 1943–45.) Starkus, A. (1947). *Gastroenterologia*, Basel, 72, 35.

The authors describe an acute fulminating form of ulcerative colitis with extremely high mortality. Bacteriological examination of the faeces showed spirochaetes of the Vincent type and fusiform bacilli. Vincent angina occurred simultaneously in a few cases. Necropsy of 130 cases showed haemorrhagic-diphtheritic inflammation with slough formation. In fulminating cases nearly the whole colon was haemorrhagic and swollen. But in a certain number the transverse colon remained free and the demarcation between the affected and non-affected parts was clear cut. Balantidia were found in one case; amoebae were not seen at all. Further proof as to the aetiology was obtained by treatment, as symptoms were usually cured by one injection of 0.3 to 0.5 g. neoarsphenamine. The whole epidemic was stopped in this way.

The author calls this clinical and pathological picture "colitis ulcerosa fuso-spirochaetosa," and considers that it should be distinguished from other types of ulcerative colitis.

The Intracutaneous Test in Cutaneous Leishmaniasis. Dostrovsky, A., and Sagher, F. (1946). *Ann. trop. Med. Parasit.*, 40, 265.

Parasites were detected in only 66.6% of patients diagnosed as suffering from cutaneous leishmaniasis, but in 261 infected persons an intradermal test was positive in 91.6%, while in 144 control patients presumed not to be specifically infected it was positive in only 6.3%. The reaction appeared to become positive within a few days of infection, and to remain so during the period of infection and for many years after the lesions had healed. Observations on 11 persons of the Prausnitz-Kuestner passive transfer test with the sera of 2 infected patients suggested that the reaction was of an allergic nature.　　　　　　　　　*F. Murgatroyd.*

Serological and Clinical Observations in Cases of Epidemic Typhus in Sweden in 1945. (Serologiska och kliniska iakttagelser vid fall av epidemisk flackytyfus i Sverige 1945.) Hammarstrom, E., Hellsten, H., and Fahraeus, J. (1947). *Nord. Med.*, 33, 700.

Serogical investigations by means of rickettsia agglutination and the Weil-Felix reaction were done on 104 cases of typhus and two control groups. The normal limits of agglutinating titre were taken as 1 in 320 for the Weil-Felix and 1 in 5120 as the upper limit of normal in the rickettsia agglutination test. A table showing the results in the 103 typhus cases suggests that the rickettsia agglutination is the more reliable diagnostic method.

Results Obtained by Streptomycin Treatment in 8 Cases of Diarrhoea with *Bacterium coli* and *Proteus* in the Stools. (Resultados obtenidos en 8 casos de diarrea con escherichia coli y proteus en las evacuaciones tratados con estreptomicina.) Aguirre, A., Aguayo, A., and Benavides, L. (1947). *Bol. méd. Hosp. infant.*, 4, 4.

Colon bacilli were found in the stools of 8 cases of severe infantile diarrhoea. *Bacterium coli-gomez* and *Proteus* were each found in 3 cases. In 7 cases tested both organisms proved susceptible to streptomycin. Intensive treatment with sulphonamides and, in 4 cases, with penicillin failed. Symptomatic treatment was maintained during the whole course of the disease. Streptomycin was used after other drugs had failed. It was injected 3-hourly, the dose and length of administration varying from case to case. Six cases recovered and 3 died.

The authors recommend a dose of 100,000 to 150,000 units of streptomycin per kilo per day, according to the severity of the case.

Streptomycin in Miliary Tuberculosis. Its Effect on the Pathological Lesions of Generalized Miliary Tuberculosis in Human Beings. Baggenstoss, A. H., Feldman, W. H., and Hinshaw, H. C. (1947). *Amer. Rev. Tuberc.*, 55, 54.

Five patients who had miliary tuberculosis were treated with streptomycin. Although the cases proved fatal, it is encouraging that in 4 cases there was convincing evidence of regression and healing of miliary tubercles in the lungs, liver, and spleen, as shown by the occurrence of fibrosis and hyalinization and the absence of caseation. Tubercle bacilli were, however, usually recoverable from the lesions. A significant concentration of streptomycin was found in the cerebrospinal fluid, in contrast to its complete absence in the brain, which helps to explain the presence of active lesions in the brain with the absence of meningitis. On the other hand, no healing was observed in kidney or bladder lesions in spite of high streptomycin concentration. No histological evidence of any toxic effect of the drug could be found, with the possible exception of renal tubular damage in one case.　　　　　　　　　*L. E. Houghton.*

Occurrence of Tuberculosis in B.C.G.-vaccinated Recruits. (Om förekomsten av tuberkulösa sjukdomar bland calmettevaccinerade värmpliktiga.) Savilahti, M. (1947). *Nord. Med.*, 33, 72.

Vaccination with B.C.G. on a large scale was first carried out in Finland during the war, when recruits were skin-tested and 13,400 negative reactors were vaccinated.

The incidence of all forms of tuberculous disease was observed over a period of 6 months to 2 years, and compared with that in a control series of unvaccinated subjects observed in 1933, while a more rigid control was obtained by a comparison of the incidence among subjects in the same call-up class who had given a positive reaction and hence were unvaccinated. Of the vaccinated subjects, 3% contracted tuberculosis within the 2-year period, one-half within the first 6 months, compared with 4% of the tuberculin-positive and 5.7% of the control group. The frequency of the various conditions is shown in the following table, the figures representing percentage incidence:

	Vaccinated subjects	Positive reactors	Controls
Pleural effusion	1.82	1.64	2.31
T.B. lymphadenitis ..	0.41	0.67	0.76
Erythema nodosum ..	0.09	0.06	0.07
Pulmonary tuberculosis ..	0.48	1.41	1.98
Other forms	0.14	0.06	0.20

Tuberculosis in vaccinated subjects was for the most part benign in character. The mortality figures per 10,000 were as follows:

	First year	Second year	Third year
Vaccinated	7.3	0	3.7
Positive reactors ..	4.6	8.5	6.5
Controls	12.2	19.2	15.7

D. J. Bauer.

B.C.G. Vaccination in Denmark in Recent Years. (B.C.G.-vaccinationen i Danmark i de senere Aar.) SLOTTVED, A. (1947). *Nord. Med.*, 33, 68.

Whereas 24 B.C.G. vaccinations were carried out in Copenhagen in 1936, the figure had risen to 8,770 in 1945. The total figures for the whole of Denmark were 133 in 1936, rising to 30,311 in 1945; 93,139 vaccinations have been carried out in all since the service started. The percentage of the population vaccinated is greatest in Bornholm (23.3% by 1945); in other places it does not yet exceed 5%.

The incidence of bovine tuberculosis has been much reduced in Denmark in recent years. In 1937 50% of the population was free from tuberculosis; in 1946, 95 to 100%. In one district studied the percentage of positive tuberculin reactors was low in the age groups below 15 to 20 years, suggesting a reduced exposure during the last few years. *D. J. Bauer.*

Rheumatoid Arthritis. IV. Hemolytic Streptococcus Precipitin Reactions. WALLIS, A. D. (1947). *Amer. J. med. Sci.*, 213, 87.

The author concludes that it is unlikely that the sera of patients with rheumatoid arthritis contain a genuine excess of haemolytic streptococcus precipitins. The antigens employed in the precipitin reactions were: (1) extracts made by Lancefield's method from haemolytic streptococci of groups A, B, C, D, E, F, and G, and (2) the C-carbohydrate substance characteristic of group A. The sera were obtained from patients with typical rheumatoid arthritis and from non-arthritic individuals free from recent streptococcal infection.

T. D. M. Martin.

The Relation Between Induced Resistance to Penicillin and Oxygen Utilization. BELLAMY, W. D., and KLIMEK, J. W. (1947). *J. Bact.*, 53, 374.

After 64 transfers a strain of *Staphylococcus aureus* had its resistance to penicillin increased over 60,000. This strain also lost its ability to grow anaerobically, while its rate of aerobic growth was from one-half to two-thirds that of the penicillin-sensitive parent strain. Under similar conditions three other organisms, *Streptococcus faecalis*, *Str. agalactiae*, and *Clostridium perfringens* developed very slight resistance to penicillin: the resistance of *Str. faecalis* was increased only 11 times in 47 transfers, that of *Str. agalactiae* 6 times in 24 transfers, and that of *Cl. perfringens* 10 times in 25 transfers. It is suggested that penicillin interferes with some anaerobic metabolism. Organisms which are unable to develop or utilize an alternative energy mechanism do not become resistant to penicillin.

G. M. Findlay.

A Mechanism for the Development of Resistance to Streptomycin and Penicillin. KLEIN, M. (1947). *J. Bact.*, 53, 463.

The actual number of bacteria employed was found to play an important part in determining the minimum and maximum effective doses of penicillin or streptomycin on any particular organism. By the passage of organisms through a medium containing progressively larger doses of the antibiotic, resistance to both penicillin and streptomycin was raised. But whereas resistance to streptomycin increased very rapidly to a high level, increased penicillin resistance developed much more slowly and at a lower level. Variants possessing very high resistance to penicillin were not found in the investigation reported. *H. J. Bensted.*

Penicillin Therapy of Subacute Bacterial Endocarditis. A Study of the End Results in Thirty-four Cases, with Particular Reference to Dosage, Methods of Administration, Criteria for Judging Adequacy of Treatment and Probable Reasons for Failures. PRIEST, W. S., SMITH, J. M., and McGEE, C. J. (1947). *Arch. intern. Med.*, 79, 333.

Of 34 patients with subacute bacterial endocarditis, 22 are alive and free from evidence of disease 13 to 35 months after completing a course of penicillin treatment. Great stress is laid on the importance of adequate dosage during treatment, 500,000 units given intravenously being the daily minimum recommended. Larger doses were employed in some cases. Daily doses of less than 400,000 units are useless. Treatment was continued for over 4 weeks; if at the end of this time the patient was not improving the dose was increased to 2,000,000 units daily.

Alan Kekwick.

The Cellular Mechanism of Recovery after Treatment with Penicillin. I. Subacute Bacterial Endocarditis. MOORE, R. A. (1946). *J. Lab. clin. Med.*, 31, 1279.

Lesions of the heart in subacute bacterial endocarditis in 22 patients treated with penicillin and in 8 patients given no specific therapy are compared. The vegetation

is composed of a central core of necrotic tissue, a layer of bacterial colonies embedded in fibrin, and a superficial thin layer of fibrin. Penicillin promotes healing in subacute bacterial endocarditis, but the basic processes of healing are not modified. These processes are: covering of the exposed surface of the vegetation with fibrous tissue, invasion of the layer of colonies and phagocytosis of bacteria, calcification of bacterial colonies, hyalinization and calcification of the central core of the vegetation, and endothelization of the spaces and clefts in the vegetation. In some patients healing is accompanied by excessive calcification and the result is a calcified stenosis of the valve. Healing in subacute bacterial endocarditis is essentially the conversion of a vegetative endocarditis into a chronic endocarditis characterized by superficial vascularized connective tissue and central nodular calcification. *T. Semple.*

Enhancement of Plasma Penicillin Concentrations by Caronamide and Sodium Benzoate. STRAUSS, E., RICHBURG, P. L., SABA, P. Z., and ALEXANDER, J. E. (1947). *J. Lab. clin. Med.*, 32, 818.

In this study 35 subjects received caronamide by mouth together with penicillin (a) by mouth, (b) by intramuscular injection in aqueous solution, and (c) by intramuscular injection in peanut oil and beeswax. A study of the effectiveness of caronamide and sodium benzoate, individually and in combination, was carried out in 8 additional subjects. When given by mouth, the combination of both caronamide and sodium benzoate resulted in a marked enhancement of plasma penicillin concentrations and yielded better results than either drug alone. The only evidence of toxicity noted was gastro-intestinal intolerance after 12 g. of caronamide by mouth in 24 hours. For the intramuscular tests the sodium salt of amorphous penicillin was used. In nearly all cases plasma penicillin concentrations were higher when caronamide was administered concurrently. The effect of 2 injections at 4-hourly intervals and of large single intramuscular injections of penicillin was not augmented by the caronamide. Sodium benzoate in 2-g. doses enhanced the plasma penicillin concentrations after multiple injections in a manner similar to that of caronamide. Results with the calcium salt of amorphous penicillin (300,000 units per ml.) in peanut oil and beeswax indicated that caronamide (1.5 g. by mouth every 2 hours for 24 hours) gave erratic augmentation effects, but in the majority of instances plasma penicillin concentrations were increased. *G. B. West.*

The Role of Coagulase in Staphylococcal Infections. SMITH, W., HALE, J. H., and SMITH, M. M. (1947). *Brit. J. exp. Path.*, 28, 56.

The association of the pathogenicity of staphylococci with their coagulase activity was suggested by circumstantial evidence and by *in vitro* investigation of phagocytosis. The authors now report investigation of this association *in vivo*. It is concluded that staphylococcal coagulase is an important factor in the pathogenicity of staphylococci, though not the only, or even the most important, factor, because it can have no direct part in the cellular necrosis occurring in characteristic focal lesions, apparently due to toxin. But toxin cannot be produced until the organism has gained a foothold, and for this, in natural infections with small numbers of cocci,

coagulase production is essential. The latter may also be important at later stages in overcoming the phagocytic response and in aiding the setting up of metastatic infection by infected emboli. There seems little prospect of immunity against staphylococci being conferred by measures directed against coagulase activity, since coagulase itself is non-antigenic. The isolation and chemical definition of coagulase are urgently necessary. *G. T. L. Archer.*

Mycosis of the Nervous System. (Micoses do sistema nervoso.) LACAZ, C. DA SILVA, DE ASSIS, J. LAMARTINE, and BITTENCOURT, J. M. TAQUES (1947). *Arch. Neuro-psiquiat.*, 5, 1.

Neuromycotic lesions can be placed in two groups—those in which there is "tumour" formation (abscess, cyst, granuloma) and those in which there is meningitis. Both forms often occur simultaneously. In view of the ambiguity of clinical findings and of findings in the cerebrospinal fluid and blood, diagnosis can rest with certainty only on the actual identification of the organism or culture. *J. J. Keevil.*

Use of Papain in Cultivation of Bacteria from the Blood. DREYFUSS, F. (1947). *Amer. J. clin. Path.*, 17, 365.

A papain-broth medium is recommended for blood cultures. The preparation of the medium is simple. It is claimed that it combines many of the advantages of saponin and trypsin; in the author's hands it appeared useful in isolating streptococci, especially of the viridans type. The growth of micro-aerophilic organisms is also favoured. Papain-broth serves as an anticoagulant, is shown to inhibit action of sulphonamides and penicillin (the latter because of a contaminating subtiloid bacillus contained in the dry powder), and has complement-fixing properties, thereby enhancing the growth of organisms. Because of its fibrinolytic action it can be employed with advantage when clot cultures are to be made. *R. Salm.*

The Evaluation of Culture Mediums for the Routine Isolation of the Gonococcus. THYER, J. D., SCHUBERT, J. H., and BUCCA, M. A. (1947). *J. vener. Dis. Inform.*, 28, 37.

A comparison is made of the efficacy of four different culture media: (1) "difco" chocolate-agar prepared with "bacto-proteose" peptone, No. 3 agar, and "bacto-haemoglobin"; (2) Peizer's horse plasma-haemoglobin agar; (3) Mueller-Hinton starch agar, as described by the authors; (4) a modification of McLeod's medium in which McLeod's phosphate-infusion agar was used as a base, enriched with plasma-haemoglobin in the same proportions as described by Peizer. The presence of the gonococcus was confirmed by Gram's stain and the sugar fermentation and oxidase tests.

With each medium 115 cultures were prepared; 95.7% of positive results were obtained with the special medium, 87.0% with the Peizer medium, 79.3% with the difco medium, and 63.5% with that described by Mueller. On 87 comparable plates in each group, each containing 300 or fewer colonies, the average number of colonies was 58.8 for the special, 39.6 for the Peizer, 37.9 for the difco, and 10.2 for the Mueller medium. *R. R. Willcox.*

BIOCHEMISTRY

Elimination of Urea, Sodium, and Chlorine in the Supersaturated Urine and According to the Intensity of Diuresis. (Las eliminaciones de urea, sodio y oloro por la orina con sobrecargas, y según la intensidad de la diuresis.) CANTERA, M. T., JIMÉNEZ DÍAZ, C., and MERCHANTE, A. (1947). *Rev. clin. esp.*, **24**, 9.

Ambard's view that urea and sodium chloride can simultaneously reach independent concentrations in urine in case of supersaturation, with dearth of water intake, is questioned. The osmotic pressure of total solids must be taken as the criterion of renal capacity, and the idea seems surprising that maximal concentration of sodium chloride should not hamper the elimination and concentration of other elements like urea. If the kidney cannot exceed a certain limit of osmotic concentration this must be so. Some recent workers have held that in the conditions mentioned chlorides and urea may vary inversely.

The authors studied this problem in normal human subjects given varying amounts of water and a constant low-nitrogen diet and ingesting excess of urea or sodium chloride. They conclude that increased ingestion of sodium chloride with low water intake causes increase of urinary volume at the cost of water from the tissues, with increased elimination of sodium and chlorine, but with retention of a part in order to economize water. There is a well-marked diminution in the output of urea. The reverse holds when urea is given in excess. The sodium-chloride concentration at a given osmotic level can only increase at the cost of urea. There is a reciprocal inverse ratio. When enough water is ingested excess of the various salts is eliminated without bringing this function into play. The saline elimination with small amounts of water is the chief determining factor of urinary volume. In certain conditions the sodium and chlorine may be unequally excreted as in pathological states, and as seen from the action of suprarenal cortex.

D. T. Barry.

Water and Salt Depletion. MARRIOTT, H. L. (1947). *Brit. med. J.*, **1**, 245, 285, and 328.

Cases of water depletion are classified into three grades: (1) *Early.*—Definite thirst is the only sign; the deficit is about 2% of the body weight, equivalent to 1.5 litres in a 70-kg. man. (2) *Moderately severe* (3 to 4 days without water).—Marked thirst, oliguria, weakness, and slight personality changes are noted, but the subject is still capable of fair effort; the deficit is about 6% of body weight (4.2 litres in a 70-kg. man). (3) *Very severe.*—Here in addition there is a marked impairment of mental and physical capacity; the deficit ranges from 7 to 14% of body weight, or 5 to 10 litres in a 70-kg. man.

The author divides the degree of salt depletion into three grades: (1) Slight to moderate, with absence of chloride in the urine (except in Addison's disease), indicating a deficit of up to 0.5 g. sodium chloride per kilo body weight; this represents a deficit of up to 4 litres of isotonic saline in a 70-kg. man. (2) Moderate to severe depletion, absence of chloride in the urine with anorexia but with a systolic pressure above 90 mm. Hg and a deficit of 0.5 to 0.75 g. salt per kilo body weight, equivalent to a deficit of 4 to 6 litres of isotonic saline. (3) Severe to very severe depletion, absence of chloride

in the urine, with apathy, stupor, and vomiting, and a systolic blood pressure below 90 mm. Hg; suggesting a deficit of 0.75 to 1.25 g. per kilo body weight, or 6 to 10 litres of isotonic saline in a 70-kg. man. The author recommends the use of a quantitative test described by Fantus (*J. Amer. med. Ass.*, 1936, **107**, 14).

In patients with inefficient kidneys incapable of excreting a concentrated urine, the daily urinary output may need to be not less than 1.5 litres. A period of 24 hours is too long for a review of output; chloride content and volume should be reported 8-hourly; the aim should be an excretion of 3 to 5 g. sodium chloride per litre in the adult and 570 ml. urine per 8 hours. Attention is called to the correct treatment of established deficiencies. In pure water depletion patients require water only, and particularly is this the case in infants whose kidneys cannot deal with a superfluity of salt. In salt depletion the wrong treatment ·consists chiefly in failure to give saline and the patient dies of oligaemic circulatory failure. Administration of water beyond that required to cover unavoidable water losses may have serious consequences. In collapse with the pylorus closed, water fills the stomach and produces vomiting, which may drown the patient whose cough reflex is gravely depressed. In salt depletion isotonic saline should be given intravenously, but as soon as chloride appears in the urine only hypotonic saline is required—0.425% for adults, 0.2% for infants—until the salt balance appears to be fully restored as indicated above in an 8-hourly period. In mixed salt and water depletion hypotonic saline is needed. In marked peripheral circulatory failure in adults the first pint should be given in 10 minutes, a second pint in 15 minutes, a third pint in 20 minutes, a fourth pint in 30 minutes, and a pint every 2 hours until blood pressure is restored to normal.

C. C. N. Vass.

Investigations of the Cerebrospinal Fluid in Cases of Rheumatoid Arthritis. SUNDELIN, F. (1947). *Amer. J. Med.*, **2**, 579.

In 141 cases of rheumatoid arthritis (38 male and 103 female) slight changes in the cerebrospinal fluid were found in the globulin-albumin quotient in 52 cases, although no considerable difference appears in total protein values. Fifteen abnormal colloid gold curves were also obtained, and 7 increased cell counts. All the patients with pathologically increased protein contents, except 1, had neurological symptoms.

Excretion of 17-Ketosteroids in Ankylosing Spondyl-arthritis and in Rheumatoid Arthritis : A Preliminary Report. DAVISON, R. A., KOETS, P., and KUZELL, W. C. (1947). *J. clin. Endocrinol.*, **7**, 201.

An investigation of the 17-ketosteroid output in the urine of patients with ankylosing spondylitis is described. In 13 male patients an average excretion of 27.3 mg. in 24 hours was found, with a range of from 19.2 to 43.7. The average value is somewhat high for the group (the average for normal males is 14 mg.). This is contrasted with the findings in 11 female patients with rheumatoid arthritis who excreted an average of 12.8 mg. in 24 hours, with a range of from 3.5 to 21.6 (average for normal females 10 mg.).

E. F. Scowen.

An Evaluation of the Urethral Smears as an Index of Androgenic Deficiency in the Male. COHEN, E. J. (1947). *J. clin. Endocrinol.*, 7, 186.

The technique of studying urethral smears in the male is described; the findings in 15 normal and 15 hypogonadal male patients were examined. No significant difference was observed between the two groups. In the normal male the administration of oestrone sulphate and the injection of oestradiol benzoate, sufficient to produce oligospermia and lowering of the 17-ketosteroid output, did not affect the urethral smear.

E. F. Scowen.

A Rapid Supra-vital Staining Method for Assessing the Viability of Human Spermatozoa. CROOKE, A. C., and MANDL, A. M. (1947). *Nature, Lond.*, 159, 749.

A method of assessing viability of human spermatozoa is described. Four or 5 drops of seminal fluid are well mixed with 1 drop of the supravital stain (50 ml. distilled water + 1 g. "revector soluble blue 706" + 1.5 g. glucose + 0.1 g. sodium chloride + 0.3 g. disodium hydrogen phosphate, hydrated + 0.005 g. potassium dihydrogen phosphate, anhydrous; the solution must be freshly prepared or made up in 1-ml. ampoules and autoclaved). The mixture is left for 2 to 3 minutes. The spermatozoa remain motile in this stain as long as they do in seminal fluid. A smear made from one drop of the mixture is dried in air and fixed in alcoholic mercuric chloride (half volume of saturated $HgCl_2$ in distilled water + half volume of absolute alcohol) for 15 to 20 seconds, dipped into alcoholic iodine (90% alcohol containing enough iodine in potassium iodide to make a straw-coloured solution) and then into 90% alcohol and allowed to dry. It is counterstained with 1% neutral red in distilled water for 15 to 30 seconds and differentiated carefully in 90% alcohol. The smear may then be examined directly by oil immersion or passed through absolute alcohol and mounted in Canada balsam.

The nuclei of dead spermatozoa stain blue or purple, while those of living spermatozoa are clear red.

Peter C. Williams.

A Study of the Metabolism in Addison's Disease. I. On Carbohydrate and Phosphorus Metabolism. [In English.] HELVE, O. (1947). *Acta med. scand.*, 127, 543.

The present investigations were carried out in 32 cases of Addison's disease and 40 controls. The fasting blood sugar in all 32 cases showed no significant deviation from the normal, and treatment with desoxycorticosterone acetate and a low potassium diet or by high sodium-chloride intake was without obvious effect on the blood sugar. A glucose-tolerance test was performed on 23 cases; its results in all were within normal limits, and in 10 cases the adrenaline-tolerance test showed little if any deviation from the normal controls.

An investigation of the total blood phosphorus and of its component fractions revealed a slight rise in inorganic phosphate, in spite of a suggestion of a fall in the total phosphorus in 6 patients. Treatment with desoxycorticosterone acetate did not affect this, and the blood-phosphorus changes in response to the administration of glucose, adrenaline, and insulin were essentially the same as in the controls. *E. F. Scowen.*

Distribution of Cholesterol, Cholesterol Esters and Phospholipid Phosphorus in Blood in Thyroid Disease. FOLDS, F. F., and MURPHY, A. J. (1946). *Proc. Soc. exp. Biol., N.Y.*, 62, 218.

The plasma cholesterol, plasma cholesterol ester, and plasma phospholipid phosphorus were found to be significantly increased in hypothyroid conditions. The lipid ratios are also altered. The plasma cholesterol ester/total cholesterol ratio and the plasma cholesterol/plasma phospholipid phosphorus ratio were significantly increased. The cell cholesterol/plasma cholesterol and the cell phospholipid phosphorus/plasma phospholipid phosphorus ratios were decreased. These values returned to normal with adequate treatment. The changes in hyperthyroidism were inconstant, the plasma phospholipid phosphorus being the only constituent which showed a significant decrease. In both hypo- and hyperthyroidism no significant change was demonstrated in the cell lipid values. *E. F. Scowen.*

Investigations of some Biochemical Changes Occurring during Treatment of Hyperthyroidism. [In English.] LUNDBAEK, K. (1947). *Acta med. scand.*, 127, 193.

This paper describes a series of investigations carried out during the treatment of 6 cases of hyperthyroidism with methyl thiouracil. A study was made of the basal metabolic rate (B.M.R.); the excretion of nitrogen, creatine, sodium, chloride, and calcium; the cell volume; serum protein, cholesterol, and uric acid. The fall in the B.M.R. began at once and continued in an almost straight line. Increase in weight occurred at a variable time and was not related to B.M.R., pulse rate, or serum cholesterol. The serum cholesterol was shown to be an unexpectedly valuable index of individual thyroid function, with a very close relation to the pulse rate and a less close relation to the B.M.R. and weight. The serum uric acid showed no uniform change, and changes in cell volume and serum protein were transient. There was an even fall in nitrogen excretion. No variation was seen in creatinine excretion. A decrease in calcium excretion occurred after a latent period of a week, but there was no constant relation to thyroid function. The effects of treatment on sodium and chloride excretion varied from patient to patient. No uniform changes were observed in the volume of urine secreted.

Raymond Greene.

The Effect of Oral Thyroid Medication upon the Prothrombin Time. SHAPIRO, S. (1946). *J. clin. Endoc.*, 6, 742.

The plasma prothrombin time was employed as a test of hepatic function in patients receiving thyroid preparations by mouth. In 16 patients taking 0.1 to 1 g. of thyroid globulin daily for periods varying from 10 to 104 days no increase in prothrombin time occurred.

R. Bodley Scott.

Study of Carbohydrate Metabolism of the Newborn and Infants on the Basis of Double Glucose and Galactose Tolerance Tests. (Beiträge zum Zuckerstoffwechsel Neugeborener und Säuglinge auf Grund Doppelbelastungen mit Glucose und Galactose.) BARTA, L., and SASS-KORTSÁK, A. (1947). *Paediat. danub.*, 1, 88.

This article records an interesting and probably unique series of sugar tolerance tests with glucose and galactose

on newborn infants, some within 12 hours of birth, and on infants from 1 to 20 months old. In the majority the results were very similar to the adult response. Galactose was usually perfectly tolerated, no increase occurring in the blood after 6 to 10 g. had been given; glucose was sometimes less perfectly tolerated. A second dose of the sugars, to evoke a Staub-Traugott reaction, gave irregular results. It is suggested that abnormally high curves are due to "functional lability" of the liver.

R. D. Lawrence.

Carbohydrate Metabolism in the Coeliac Syndrome. EMERY, J. L. (1947). *Arch. Dis. Childh.*, **22**, 41.

The absorption of glucose has been studied in 13 cases of the coeliac syndrome and controls. In the coeliac syndrome the oral glucose tolerance curve was flat, but it was more normal if larger doses of glucose were ingested. After insulin injection the immediate fall in blood sugar was similar in the coeliac and control groups, but recovery was usually delayed in the coeliac cases. The recovery rate was not affected by administration of adrenaline, but was improved by the ingestion of glucose. The administration of adrenaline caused a rise in blood sugar, which was less than normal in the coeliac group unless glucose was simultaneously administered by mouth, when an immediate and rapid rise in blood sugar occurred in both groups. The administration of adrenaline after insulin produced little effect on the blood sugar in the coeliac patients studied. The intravenous glucose tolerance tests were within normal limits in all cases.

It is concluded that glucose absorption occurs normally in the coeliac syndrome, on the basis of the improvement in the oral glucose tolerance curve with increasing dosage of glucose and the response to oral glucose during insulin hypoglycaemia. The normal response following glucose and adrenaline indicates that the rate of absorption is normal. The flat oral glucose tolerance curve is attributed to more rapid fixation of glucose, presumably in the liver, since the intravenous glucose tolerance curves were normal. The possibility that the underlying fault may be a deficiency of liver glycogen is discussed.

A. C. Frazer.

Mauriac's Syndrome (Retardation of Growth with Hepatomegaly and Disturbance of Fat Distribution in the Diabetic Child) and its Relation to Von Gierke's Disease. (Le syndrome de Mauriac retard de taille avec hepato-mégalie et troubles de la répartition des graisses chez l'enfant diabètique et ses rapports avec la maladie glycogénique de Van Creveld-van Gierke.) HOUET, R. (1947). *Ann. Pœdiat., Basel*, **168**, 113.

This paper deals with the syndrome first described by Mauriac. Comparisons are drawn between this condition and von Gierke's syndrome, a type of hepatomegaly described a few years before Mauriac's original communication. In Mauriac's syndrome, as the present author admits, biochemical changes are not very numerous or consistent, and much further investigation is needed. Nevertheless, it seems fairly clear that the biochemistry in this group is quite different from that of von Gierke's disease and resembles that of the true diabetic.

No definite conclusion is reached regarding the aetiology. The author thinks that the problem is likely to remain unsolved until the disease can be reproduced in animals. *Patrick Mallam.*

Spontaneous Hypoglycemia with Special Reference to the Diagnosis of Hyperinsulinism. [In English.] LUFT, R. (1947). *Acta med. Scand.*, **127**, 65.

Fifteen normal subjects underwent a 3-hour intravenous glucose tolerance test and an intravenous insulin tolerance test, and 32 subjects the Exton-Rose two-dose 1-hour glucose tolerance test; the limits of the normal were thence defined in terms of mean and standard deviation for each sampling time.

Six patients with spontaneous hypoglycaemia were studied by these tests; of these, 2 had an islet-cell adenoma of the pancreas, and 1 an islet-cell carcinoma with many metastases; in the other 3 "reactive" hyperinsulinism was considered to be present. In all cases the intravenous glucose tolerance test gave low values, and hypoglycaemic symptoms appeared within 90 minutes of the start. In the intravenous insulin tolerance test the 3 organic cases showed low values of blood sugar, which failed to rise towards fasting level; the 3 "reactive" cases gave results within the normal limits, though all 6 showed marked symptoms of hypoglycaemia.

G. Discombe.

Chronic Subclinical Impairment of the Liver. Early Diagnosis and Treatment. Further Improvement and Evaluation of Certain Liver Function Tests. MATEER, J. G., BALTZ, J. I., STEELE, H. H., BROUWER, S. W., and COLVERT, J. R. (1947). *J. Amer. med. Ass.*, **133**, 909.

These authors describe eleven different liver function tests and give what they consider normal values for them. It is claimed that by using the three most delicate tests (cephalin flocculation, thymol turbidity at pH 7.55, and a new bromsulphthalein test) cases of subclinical liver damage can be detected. The authors indicate which combinations of tests they advise in various types of liver disease.

The detection of subclinical liver damage is regarded as important, for these workers maintain that the treatment of such cases with a high-carbohydrate, high-protein, low-fat diet (C 350 to 400 g.; P 125 to 150 g.; F 35 g.) with added vitamins (A and B complex) causes the sensitive tests to return to normal and prevents the development of permanent damage or cirrhosis.

C. Hardwick.

Pancreatic Secretion in Hepato-biliary Diseases. (La sécrétion pancréatique au cours de certaines affections hépato-biliares.) HERFORT, K. (1947). *Gastroenterologia, Basel*, **72**, 51.

Chronic pancreatitis is usually secondary to gall stones or cholecystitis. The author studied the pancreatic secretion in cases of liver damage caused by cirrhosis, infective hepatitis, and acute atrophy of the liver. He had previously examined the external secretion of the pancreas in 60 cases of "cholecystopathy." In 60% of the cases involvement of the pancreas manifested itself clinically and in laboratory tests. In one-quarter of his cases he could not prove involvement of the pancreas. The participation of the pancreas in liver affections has not been much studied.

In 20 patients with cirrhosis, 12 males and 8 females, examination of pancreatic external secretion showed that lipase is the enzyme whose secretion is first diminished much before that of trypsin. Lack of lipase could be

shown by examination of the faeces in 3 cases only. It is well known that the splitting of fats can occur through action of intestinal bacteria to such a degree that no deficiency of pancreatic lipase in the faeces can be detected, although disturbance of the pancreatic secretion can be proved. In 9 out of 20 cirrhotic patients pancreatic secretion was seriously disturbed. The deficiency of lipase was most marked, in contrast with the even decrease in pancreatic enzymes in cases of tumour. The degree of the pancreatic insufficiency is not parallel with the severity of the cirrhosis.

Twenty-three cases of infective hepatitis were examined; 8 showed disturbed pancreatic function, 4 had considerable insufficiency after the disappearance of the jaundice. Two cases of acute yellow atrophy of the liver after cincophen administration were examined. The secretion of duodenal juice was normal after secretion injection. Both patients died and the histology of the pancreas showed no abnormality.   *E. Forrai.*

Observations on the Diurnal Excretion of Urobilinogen in the Urine of Normal Subjects and of Patients with Laënnec's Cirrhosis. PELLEGRINO, E., PATEK, A. J., COLCHER, A., and DOMANSKI, B. (1947). *J. Lab. clin. Med.*, 32, 397.

Urobilinogen excretion was determined at three periods in the day in 39 healthy subjects and in 36 patients with cirrhosis of the liver. The mean urobilinogen excretion per hour in the urine was 0.3 Ehrlich units for normal subjects and 0.6 units for patients with cirrhosis. The period of maximum excretion varied in different subjects and in the same subject at different times. There was poor correlation between the degree of urobilinogen excretion and the clinical severity of the liver disease.
                                                   R. B. Lucas.

The Assessment of Liver Damage Following Trichlorethylene and Di-ethyl Ether Anæsthesia. ARMSTRONG, D. M. (1947). *Anæsthesia*, 2, 45.

Assessment of liver damage by the cephalin-cholesterol test showed severe damage by di-ethyl ether and less damage after trichlorethylene anaesthesia.

Biochemical Studies During Malarial and Artificial Fevers. GALL, E. A., and STEINBERG, A. (1947). *J. Lab. clin. Med.*, 32, 508.

Preliminary studies on 18 malarial patients had shown the development of a transient hypophosphataemia during febrile paroxysms. Hyperglycaemia is first produced by the accelerated metabolism during fever. Hexosephosphate is formed and deposited in the tissues, thus causing hypophosphataemia. At the end of the febrile episode the hexosephosphate dissociates and the amount of inorganic phosphorus in the serum returns to normal.   *J. E. Page.*

Postacidotic State of Infantile Diarrhea : Symptoms and Chemical Data. Postacidotic Hypocalcemia and Associated Decreases in Levels of Potassium, Phosphorus and Phosphatase in the Plasma. RAPOPORT, S., DODD, K., CLARK, M., and SYLLM, I. (1947). *Amer. J. Dis. Child.*, 73, 391.

A report is made on the signs, symptoms, and chemical changes in infants with diarrhoea during acidosis and after recovery from acidosis. The authors recognize

two phases in the illness: "(1) the phase of acidosis and dehydration, during which losses of extracellular and intracellular ions and fluids occur, and (2) the postacidotic phase, during which, after correction of deficits of extracellular ions, depletion of the nonextracellular ions ensues because of avid uptake of these ions by soft tissues and bone. Plasma depletion of these ions is attended by pronounced signs and symptoms."
                                           Eveline A. Bishop.

HAEMATOLOGY

Folic Acid in Pernicious Anaemia. Its Effect as Shown by Serial Sternal Punctures. LEVY, H. (1947). *Brit. med. J.*, 1, 412.

Serial marrow punctures on a patient with Addisonian pernicious anaemia revealed as rapid a reversion to normoblastic erythropoiesis as has been shown to occur after liver therapy. Abnormal granulopoiesis persisted for at least 48 hours after the beginning of treatment.

Relative Clinical and Hematologic Effects of Concentrated Liver Extract, Synthetic Folic Acid and Synthetic 5-Methyl Uracil in the Treatment of Macrocytic Anemias in Relapse. FROMMEYER, W. B., and SPIES, T. D. (1947). *Amer. J. med. Sci.*, 213, 135.

An important paper. The authors compare the therapeutic effects of concentrated liver extract, synthetic folic acid, and 5 methyl-uracil, using the same patients in successive relapses. Liver extract seemed to be slightly more effective than folic acid; 5 methyl-uracil was much less useful.

Nonutilization of Conjugated Folic Acid in Pernicious Anemia. HEINLE, R. W., NELSON, E. M., NELSON, H. V., and WELCH, A. D. (1947). *J. Lab. clin. Med.*, 32, 336.

Folic acid conjugate (heptaglutamate) was ineffective when administered to three patients with Addisonian anaemia. Two of the patients subsequently responded to synthetic folic acid.

The Treatment of Pernicious and Related Anemias with Synthetic Folic Acid. I. Observations on the Maintenance of a Normal Hematologic Status and on the Occurrence of Combined System Disease at the End of One Year. VILTER, C. F., VILTER, R. W., and SPIES, T. D. (1947). *J. Lab. clin. Med.*, 32, 262.

This work provides further evidence that folic acid is ineffective in controlling the neurological complications of Addisonian pernicious anaemia, and that reliance upon it in the maintenance treatment of this disease is to be deprecated. It confirms the view, however, that folic acid is effective as a haematinic in megaloblastic anaemia, and that it may be of definite clinical value in cases associated with intestinal deficiency.
                                                   L. J. Davis.

Atypical Anemia, with Spherocytes and Target Cells Coexisting in the Blood. DISCOMBE, G., and WATKINSON, G. (1947). *Amer. J. med. Sci.*, 213, 153.

An interesting report.

A Study of the Sternal Marrow and Peripheral Blood of Fifty-five Patients with Plasma Cell Myeloma. DIGGS, L. W., and SIRRIDGE, M. S. (1947). *J. Lab. clin. Med.*, **32**, 167.

Stilbamidine and Pentamidine in Multiple Myeloma. SNAPPER, I. (1947). *J. Amer. med. Ass.*, **133**, 157.

Stilbamidine and/or pentamidine administered intravenously resulted in clinical remission with alleviation of pain. The course of the disease appeared to be checked. These substances form compounds with ribonucleic acid in the cytoplasm of the myeloma cells. Deeply basophilic inclusions develop.

The treatment was combined with a diet low in protein by American standards: the specimens given provide about as much protein as the current British rations.

Icterus Neonatorum : Its Incidence and Cause. FINDLAY, L., HIGGINS, G., and STANIER, M. W. (1947). *Arch. Dis. Childh.*, **22**, 65.

The authors conclude that "physiological" jaundice in the newborn is more likely to be due to hepatic immaturity than to haemolysis.

The Influence of Heat and Formalin Upon the Rh Agglutinogen. LUBINSKI, H. H., and PORTNUFF, J. C. (1947). *J. Lab. clin. Med.*, **32**, 178.

After 5 to 20 minutes heating at 50° C. red blood cells are not, or hardly, agglutinated by anti-Rh agglutinating and blocking sera, but normal agglutination occurs with anti-A, anti-B, anti-M, and anti-N sera. Agglutination of red-cell suspension, after treatment with dilute formalin (0.1 to 1%), is inhibited to a greater extent for anti-Rh serum than for anti-A and anti-B sera of equal titre. This latter effect is not due to haemolysis of the red cells. The reason may be due to: (1) the Rh agglutinogen is situated on the surface of the cells and the others within the cells; (2) the Rh agglutinogens may be less numerous and are, therefore, destroyed more quickly; or (3) there may be a difference of chemical structure of the different agglutinogens.   *John F. Wilkinson.*

An Anticoagulant Present in the Blood of a Clinically Haemophiliac Patient. (Sur un anticoagulant présent dans le sang d'un sujet cliniquement hémophile.) LAMY, M., BURSTEIN, M., and SOULIER, J. P. (1946). *Rev. hémat.*, **1**, 421.

In a patient suffering from the clinical manifestations of haemophilia the blood plasma delayed the clotting of normal blood or plasma. Examination showed normal bleeding time, tourniquet test, capillary fragility, blood count, platelet count, prothrombin time, clotting on addition of thrombin, and content of "masked" antithrombin; the anti-fibrinolytic power of the blood was normal. When 1/10 to 1/20 volume of the patient's non-citrated plasma, unheated or heated to 65° C. for 30 minutes, was added to normal blood or to normal plasma, which was then recalcified, the clotting time was prolonged 2 to 18 times. The clotting time of the patient's recalcified citrated plasma varied with the content of platelet substance: it was greatly prolonged when plasma freed of platelets by centrifugation and filtration was used, less prolonged when platelets were normal in number and intact, and least (though still

greater than normal) when the platelets were destroyed by freezing or by addition of distilled water; it was not affected by addition of protamine.

The authors conclude that a circulating anticoagulant was present; it was neither heparin, an anti-prothrombin, nor an anti-thrombin; it probably interfered with the activation of the thromboplastin of plasma or platelets.
                                   G. Discombe.

MORBID ANATOMY AND HISTOLOGY

Further Notes on the Pathology of Acute Epidemic Hepatitis and Homologous Serum Jaundice. WOOD, D. A., and BLACK, M. B. (1946). *Amer. J. clin. Path.*, **16**, 746.

The pathology was studied at necropsy in 8 patients dying from 2 to 10 days after the onset of hepatitis, most of the cases being of homologous serum jaundice. On the second day of jaundice the liver cells were swollen and detached. Inflammatory infiltration followed. Patients dying on the seventh day showed mid-lobular necrosis. A case is reported of a child, aged 3¼ months, who died on the eighth day of what was thought to be homologous serum jaundice following transfusion after birth for a suspected mild erythroblastosis. The liver showed diffuse subtotal necrosis and autolysis of liver cells infiltrating the stroma.   *M. Le Vay.*

The Pathogenesis of Polycystic Livers. Reconstruction of Cystic Elements in Two Cases. NORRIS, R. F., and TYSON, R. M. (1947). *Amer. J. Path.*, **23**; 201.

This article describes the reconstruction of the cystic elements in portions of liver from 2 newborn infants. The livers had a normal contour, but microscopical examination revealed irregular cystic dilatations of the intrahepatic bile ducts, which were lined by cubical epithelium and encircled the branches of the hepatic artery and portal veins. The models, reconstructed from serial sections, show numerous anastomoses and irregular dilatations of the intrahepatic bile ducts, some of which were cut off into isolated segments. The authors consider that the lesions result from an abnormal extension of the normal processes of degeneration in the bile ducts, causing distension, segmentation, and cystic dilatation, while normal differentiation of the hepatic anlage occurs elsewhere.   *E. T. Ruston.*

Liver Biopsy in Thyrotoxicosis. [In English.] PIPER, J., and POULSEN, E. (1947). *Acta med. scand.*, **127**, 439.

Liver-function tests were performed in 30 cases of thyrotoxicosis; liver biopsy was carried out in 15. In 20% of cases the Takata-Ara test was positive. An increase in the serum phosphatase of 11 or more Buch units (normal value 0 to 7 Buch units) occurred in 9 out of 28 patients. There was no significant alteration in the formol-gel test, prothrombin time, plasma colour, platelet count, serum iron and urine urobilin estimation. No relation between the metabolic rate and the results of liver-function tests could be demonstrated. The 15 liver biopsies showed normal liver tissue 5 cases, glycogen (picric-acid fixation) markedly reduced in 2 cases, slight steatosis in 3 cases, delicate dark streaks of cells in 5 cases, slight round-cell infiltration in 2 cases, and suspected commencing cirrhosis in 1 case. The authors point out that a single negative biopsy result cannot exclude the

possibility of more pronounced changes in the liver, but the peripheral zone is the most likely to show changes, and that is the zone examined in needle biopsy.

S. Oram.

Combined Anterior Pituitary Necrosis and Symmetrical Cortical Necrosis of the Kidneys following Accidental Haemorrhage. GRASBY, E. D. Y. (1947). *J. Obstet. Gynaec. Brit. Emp.*, 54, 203.

Three cases of accidental uterine haemorrhage are described. In the first, necropsy revealed combined renal cortical necrosis and pituitary necrosis; in the second there was cortical necrosis with clinical and pathological evidence of less renal destruction than is usually seen; in the third there was probable recovery from cortical necrosis.

Epidemic Poliomyelitis. Some Pathologic Observations on Human Material. LUHAN, J. A. (1946). *Arch. Path.*, 42, 245.

A detailed histological study of the central nervous system was made in 13 fatal cases from the 1943 Chicago epidemic of poliomyelitis. Duration of illness was 3 to 21 days, the mean being 6 days; clinically all cases showed evidence of bulbar involvement. Slight or moderate cerebral oedema was found in 12 cases. Blocks of tissue were examined histologically from eleven representative areas in the cerebral cortex; the typical changes were confined to the motor cortex in all but 1 case, this agreeing with findings of other workers. The olfactory bulbs and tracts were available for examination in only 5 cases; all were normal. Sections of medulla oblongata and cervical cord showed the inflammatory reaction to be more intense here than elsewhere ; a table is given in which numerical estimates have been made of the intensity of the reaction in 14 different sites in the central nervous system. In 5 cases of 9 in which sufficient material was available to permit the comparison the most recent process (as evidenced by the large number of polymorphonuclear leucocytes present) was found in the lumbar, sacral, and lumbo-sacral segments. From a consideration of the clinical features and histological findings the author classifies his cases into 9 primary "bulbar" types, 3 primary spinal types, and 1 indeterminate. *W. S. Killpack.*

Amyloid Macroglossia. Report of a Case. BABER, M. D. (1947). *Lancet*, 1, 210.

A man of 63 had swelling and induration of his tongue causing difficulty in swallowing for about 6 weeks. He died from coronary thrombosis after an unsuccessful attempt had been made to insert an oesophagoscope. Necropsy revealed amyloid infiltration of the tongue, the buccal mucosa, the oesophagus, and the heart. The oesophagus was a thickened almost rigid tube from amyloid infiltration of its wall. The liver and spleen were normal, but there were a few deposits of amyloid substance in the glomerular tufts of the kidney. The condition differed from the classical form of amyloid disease which follows protracted suppuration, tuberculosis, and syphilis, and which commonly affects the liver and spleen. *J. B. Duguid.*

Human Toxoplasmosis. A Clinicopathologic Study with Presentation of Five Cases and Review of the Literature. CALLAHAN, W. P., RUSSELL, W. O., and SMITH, M. G. (1946). *Medicine, Baltimore*, 25, 343.

About 36 cases of human toxoplasmosis have been recorded, but there is reason to believe that the incidence is considerably higher and that it has a wide geographical distribution. This view is based on the detection of unsuspected infections in 60 out of 250 persons in the United States by means of the "neutralization test." The authors have critically examined and analysed the necropsy material and records of 10,000 cases in the department of pathology, Washington University School of Medicine, St. Louis. This search revealed 5 cases listed in the records under "chronic meningitis," "syphilitic meningitis," and "encephalomalacia with calcification." A clinico-pathological study of these cases, coupled with the finding of the causative organism in all of them, left no doubt that they represented undetected instances of toxoplasmosis.

Anuria Following Criminal Abortion. YOUNG, J., and WALKER, A. H. C. (1947). *J. Obstet. Gynaec. Brit. Emp.*, 54, 196.

The authors describe a case of widespread uteroplacental injury followed rapidly by oliguria, azotaemia, and death. The patient, aged 31, single and nulliparous, was admitted after amenorrhoea lasting $11\frac{1}{4}$ weeks. Nine hours before admission an abortionist had injected 12 oz. of "dettol," soap, and water into the uterus with an adapted Higginson's syringe. From the time of the injection to the patient's death ($5\frac{3}{4}$ days) only $8\frac{1}{4}$ oz. of urine was excreted. There was no oedema. During that time the blood pressure rose from 90/75 to 140/90 mm. Hg, the blood urea from 115 mg. to 290 mg.% and the serum potassium from 23.6 mg. to 37 mg.%, while the plasma chloride fell from 540 mg. to 473 mg.%. The urine contained albumin and a normal concentration of urea.

At necropsy the placenta was found closely applied to the anterior wall of the uterus, but the membranes were separated from the posterior wall by gelatinous material containing fatty acids and smelling of dettol. This material reached to the upper edge of the placenta, the neighbouring 3 to 4 cm. of which was haemorrhagic and friable. The uterine wall in this area was pale, soft, and friable. The upper posterior uterine wall showed typical infarction, with vessels completely occluded with granular debris. The ovary contained a large corpus luteum, and there was enormous dilatation of veins and capillaries, with widespread interstitial haemorrhage. The foetus corresponded to 16 weeks of gestation. The classical picture of "transfusion" or "crushing injury" kidney was seen with pigmented debris in the tubules giving a positive benzidine reaction for haemoglobin. The pituitary showed a focus of necrosis in the pars anterior.

The authors consider that the soap and dettol injected under pressure probably entered the large veins of the uterus, and that much of the necrosis was the result of the direct action of the chemicals. The extensive haemorrhage was possibly due to the thrombosis of the main vascular channels. They point out the marked similarity of uterine and renal lesions in their case to those seen in concealed accidental haemorrhage. The renal lesions resemble those found after crushing injuries, extensive

burns, and "mismatched" blood transfusions, the common factor being the presence of extensive tissue damage. The blood transfusions could not be incriminated in this case, for the profound oliguria had begun before it was given. The anuria in this type of case is due to cortical ischaemia, and tubular blockage from blood casts and debris, with probably a direct toxic effect on the tubules. This cortical ischaemia has recently been ascribed to a short circuit of the total renal blood flow through the medullary vessels (Trueta *et al.*, *Lancet*, 1946, 2, 237). Similar, but less severe, renal lesions, with pigment deposits, may be found in cases of eclampsia, and the authors consider them to be due to the massive placental damage found in such cases.   *Aileen M. Dickins.*

A Cause of Rapid and Unforeseen Death of Infants : Milk Embolism in the Lung. (Sur une cause de mort rapide et imprévue du nourrisson: l'embolie de lait dans le poumon.) MARIE, J., SERINGE, P., and HÉBERT, S. (1947). *Sem. Hôp., Paris*, 23, 1335.

The author thinks that in the 3 cases described the deaths of the infants were attributable to inhalation into the air passages of milk regurgitated from the stomach.

In each case the lungs showed histological evidence of patchy bronchopneumonia of very acute type, distinguished by the presence in bronchioles and alveoli in the affected areas of foreign material staining readily with fat stains. Such a histological picture could be exactly reproduced by the intratracheal injection into a guinea-pig of a few ml. of milk, the animal being killed 15 minutes after this procedure. Evidence of a vital reaction to the foreign material (compensatory emphysema, cellular reaction, and alveolar exudate) in the affected bronchopulmonary segments led the authors to conclude that the milk had found its way into the bronchial tree during life, and that its presence there was not due to post-mortem regurgitation. They suggest that this event is a not uncommon cause of sudden death in apparently healthy infants. In some cases reflex laryngeal spasm brought about by intratracheal inhalation may be sufficient to cause death from asphyxia. They advise, therefore, that babies under 2 months old should not lie horizontally in their cots, especially if they have a tendency to vomit, but should be propped semi-vertically with pillows.   *M. MacGregor.*

Lesions of the Central Nervous System in Two Cases of Kernicterus. (Lésions du système nerveux central dans deux cas d'ictère nucléaire du nouveau-né.) BERTRAND, I. (1946). *Rev. hémat.*, 1, 399.

The distribution of bile-stained areas and the histological changes found in the brains of 2 infants who died from kernicterus are described. The highest degree of bile staining was found in the amygdaloid nucleus, the thalamus, the hippocampal gyrus, the optic radiation, the depths of the occipito-parietal sulcus, the nuclei of the cranial nerves III to VIII inclusive and XII, all structures in the floor of the fourth ventricle, the olive, the dentate nucleus, especially the dorsal lamina, and the whole granular layer of the cerebellum. Less severe staining was found in the grey matter of the anterior perforated space, caudate and lenticular nuclei, body of Luys, superior corpora quadrigemina, and small areas on either side of the interparietal, superior frontal, and collateral sulci.

Histological changes were not confined to the bile-stained areas. They consisted of oedematous degeneration of the nerve cells affecting most of the cortex, with patches of more severe degeneration irregularly distributed but most marked in the bile-stained areas, and particularly affecting the dentate nucleus and the nuclei of the third and sixth nerves, which could hardly be identified because of the destruction of nerve cells. There was only slight glial reaction, with irregular perivascular infiltration of mild degree.

        G. Discombe.

Multiple Myeloma. Review of Eighty-three Proved Cases. BAYRD, E. D., and HECK, F. J. (1947). *J. Amer. med. Ass.*, 133, 147.

Cytochemical Differentiation between the Pentose and Desoxypentose Nucleic Acids in Tissue Sections. SAUNDERS, F. K. (1946). *Quart. J. micr. Sci.*, 87, 203.

The intracellular distribution of the two kinds of nucleic acid is ascertained by comparing the enzyme-treated sections with their controls. Staining with celestine blue indicates the presence of desoxyribonucleic acid, and staining with pyronin that of the pentose acid. The results of the technique are illustrated by reference to the Purkinje cells of the guinea-pig. The specificity of the reactions is discussed, and it is concluded that the method provides a satisfactory means of distinguishing the two kinds of nucleic acid in fixed tissues.

        A. K. Powell.

Cancer Cells in Prostatic Secretions. HERBUT, P. A., and LUBIN, E. N. (1947). *J. Urol.*, 57, 542.

Urine obtained after digital massage of the prostate was examined for cancer cells. A diagnosis of carcinoma was made on cytological grounds in 17 of 100 cases. In 10 the diagnosis was confirmed histologically. In 7 it was not confirmed, but in 6 it was thought to be reasonably certain on clinical grounds. The authors think the results warrant further investigation.

NORMAL AND ABNORMAL BLOOD COAGULATION: A REVIEW

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Introduction

As one of the lesser problems of physiology, the nature of blood coagulation has received more than its share of investigation. There is now an insurmountable mass of literature dealing with its various aspects, contributed over the greater part of a century by some of the foremost experimentalists of their time, and embodying theories, even whole schools of thought, that have produced little but acrimony and confusion. The reasons for this activity are not at once apparent, though no doubt there is something provocative to the curious mind in the spontaneous conversion of fluid blood to solid clot. More attractive, perhaps, is the ease with which experiments in blood coagulation can be devised and carried out. The requirements for such work in technique, apparatus, and even in time are seemingly not exacting. The real or imaginary components of a theoretical mechanism can be separated to the investigator's satisfaction with little difficulty and allowed to interact in endless permutations and combinations. Each experiment suggests another ; always the intangible solution seems just within reach and the experimenter is led deeper and deeper into his own, often unjustified, interpretations of his findings. Thus it is that elaborate theories have grown up which, aided by the confusion of rival terminologies, have so obscured and entangled basic facts that these are now scarcely recognizable.

Within the last decade this hitherto rather academic problem has taken on a more practical aspect ; the discovery of vitamin K and the use of anticoagulants to reduce the danger of post-operative thrombosis, both advances of fundamental importance, have demanded the reliable quantitative assay of coagulation factors. This development has necessarily clarified

some of the obscurities, but it has thrown a strain on accepted theory, which is now showing signs of undergoing considerable modification. It seems, therefore, that some review or rather " interim report " of the problem might be desirable. Though the present communication is an attempt to provide this, it is no more than an outline and in no way claims to be comprehensive.

The Function of Blood Coagulation

Haemostasis.—Before discussing the mechanism of blood coagulation, it would be well to consider briefly its functional significance. Its most obvious use is concerned with haemostasis. The fact that wounds which have ceased to bleed are filled with blood clot naturally suggests that the clotting of the blood has arrested the haemorrhage. This is supported by the fact that in conditions with defective blood clotting there is a tendency for patients to bleed for long periods from even slight injuries. Coagulation is not, however, the only factor concerned in this tendency, since severe haemorrhagic states may exist despite a normal clotting mechanism. The part played by coagulation has been discussed in detail by the author (Macfarlane, 1941), who concluded that after injury there is a period of bleeding from cut vessels which is succeeded by a period of haemostasis lasting for about an hour and which is due primarily to the contraction of the damaged vessels. These vessels then dilate, but recurrence of the haemorrhage is prevented by the firm coagulation of the blood that had filled the wound during the initial haemorrhage. Normal coagulation, therefore, is the essential complement to the vascular contraction (and perhaps normal platelet function) which initiates haemostasis. Either factor without the other is useless. This " two stage " hypothesis of haemostasis and the two main abnormalities which, in the author's view,

1

cause the haemorrhagic states is illustrated diagrammatically in Fig. 1 (Macfarlane, 1945). It will be seen that this hypothesis in no way lessens the supposed importance of coagulation in the mechanism of haemostasis, but regards it as an interlocking part of a more complex system.

	Immediate	2 minutes	20 minutes
Normal			
Coagulation Defect			
Capillary Defect			
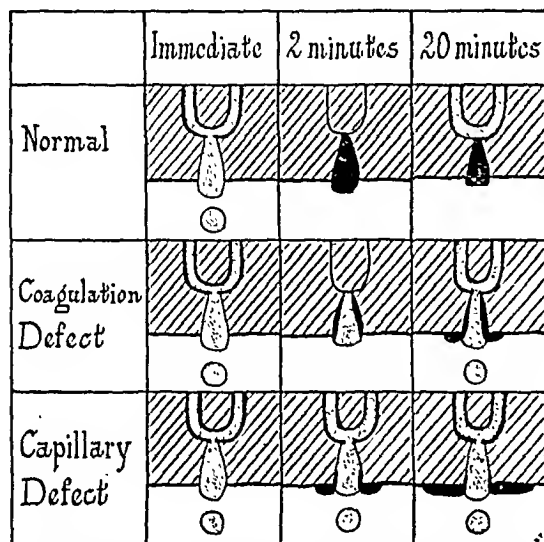
Fig. 1.—Diagram illustrating the time relationship of normal capillary contraction, dilatation, and blood coagulation, and the two main defects that may occur. A wound of the skin surface is shown in section, injuring a capillary loop. Fluid blood is represented by the dotted areas, blood clot by solid black, and the detached "drops" indicate active haemorrhage. (Macfarlane, 1945. Reproduced by permission of the publishers of the *Proc. roy. Soc. Med.*)

Resistance to bacterial invasion.—The inflammation accompanying local bacterial infection is usually associated with plasma transudation into the tissue spaces. Coagulation of this oedema fluid takes place and the fibrin so formed serves as a barrier more or less effective in preventing the spread of infection through the adjacent tissues. It has been found experimentally (Thuerer and Angevine, 1947) that impairment of coagulation by administration of dicoumarin greatly reduces the minimal lethal dose of pathogenic organisms injected subcutaneously into rabbits. It may be that the characteristic invasiveness of the haemolytic streptococci as compared with the localization of staphylococcal infection is due to the fibrinolytic action of the former as against the coagulase produced by the latter. It has been pointed out by Robb-Smith (1945) that a feature of gas-gangrene due to *Cl. welchii* is the almost complete absence of local fibrin formation, and in no other infection is tissue invasion so rapid. The fact that in most infections there is a marked

rise in the fibrinogen content of the blood may be related to a defence reaction of this type.

Healing.—The importance of a fibrin network in the process of repair of wounds has often been suggested, but actual proof is not available. It is a fact, however, that in haemophilia the healing of even minute wounds is extremely slow if haemorrhage is taking place. It is no unusual experience for a patient to ooze blood for weeks from a small abrasion that normally would be healed completely in a few days. The continued bleeding itself may prevent the reparative processes from making headway, but it is also possible that these require the presence of preformed fibrin as a scaffold for the new network of collagen and capillaries.

Metabolism.—The mechanism of blood coagulation is extremely elaborate, requiring the interaction of many factors. Some of its protein constituents, such as fibrinogen and prothrombin, are normally present in considerable excess of what is required for haemostatic efficiency. This prodigality has seemed to a number of authorities to suggest that coagulation is a result of a constantly operating metabolic process that has been adapted to fulfil the secondary purpose of haemostasis. In support of this, it appears that both fibrinogen and prothrombin are consumed with considerable rapidity in the absence of any obvious clotting activity, though some of the evidence from which this is inferred is questionable, as will be seen later. The fact that proteolysis appears to be in some way connected with the coagulation mechanism also suggests that the latter may be concerned in normal protein metabolism.

Finally, despite these speculations it can be said that blood coagulation is not essential to life. Several patients have been observed to lead a relatively normal existence though they have no fibrinogen in their blood.

Normal Blood Coagulation

Historical.—While it is outside the scope of this article to give more than an outline of the historical background to the present views on blood coagulation, such a background assists the visualization of the problem in its proper perspective.

Malpighi (1666) was one of the first to consider the nature of the solidity of clotted blood. He found that after a clot was washed in water a mass of fibres remained. In 1735 the French surgeon Petit observed that coagulation was an attribute of the clear, fluid part of the blood, not of the cells, an observation that was extended by Hewson (1770), who called this fluid "coagulable lymph" and showed that clotting could be prevented by cold, or the presence of salts.

Chaptal (1797) applied the appropriate name, "fibrin," to the fibres constituting the clot.. The nature of the clotting process then began to evoke considerable discussion. Hunter (1794) maintained that it was a "vital" action of the blood. analogous to the contraction of a living muscle. Others considered that it was chemical in nature, and depended on such changes as contact of the blood with the air, cooling, or stasis.

In 1835. Buchanan made a series of observations that anticipated the discovery of thrombin and thromboplastin. He showed that the clotting of "coagulable lymph," in this case hydrocele fluid, was accelerated by the addition of the washings of a blood clot or of leucocytes. He compared the action of these substances to that of rennin on milk, thus founding the concept of a reaction between a precursor of fibrin and a ferment-like agent generated in the blood during clotting; but his work received little recognition until it was resuscitated by Gamgee (1879). The development of a clearer understanding of the coagulation mechanism was hampered for a while by the popular theory, propounded by Richardson (1858), that blood clotted when it left the vessels because the ammonia it contained during life was then able to escape. This tenacious fallacy was finally exploded by Lister (1863), who performed a series of experiments that are a model of ingenuity and precision. A few years before this Denis (1859) had been able to separate the fluid precursor of fibrin, calling it "plasmine," a name changed to the more descriptive "fibrinogen" by Hammarsten (1877).

The scene was now set for Schmidt (1892) to present his conception of a chain of reacting factors culminating in the conversion of fibrinogen into fibrin by a ferment called thrombin, a theory that has held the stage ever since. Schmidt recognized that thrombin was not present in the circulating blood, and he postulated an inactive precursor, prothrombin, that was activated by zymoplastic substance, considered, even at that early stage, to be lipoidal in nature.

The hypothesis of coagulation, as it stood at this time, is illustrated by the following diagram quoted by Quick (1942):

Cells
|
Plasma
|
Cytoglobulin   Zymoplastic———→Prothrombin
                Substance
Paraglobulin |
| |
Fibrinogen ←————————————————Thrombio
|
Soluble Fibrin+Salts = Fibrin
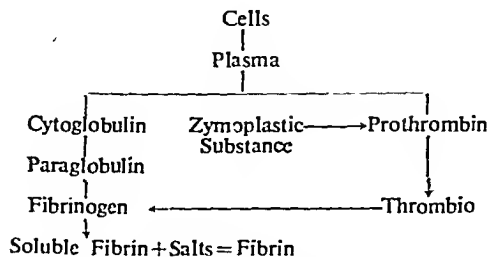
It remained for Arthus and Pagès' (1890) discovery of the essential part played by ionized calcium in the activation of prothrombin to complete the basis for the modern conception of the coagulation mechanism. This was restated in a more simple form by Morawitz

(1905), who considered that clotting involved two stages and four essential components, thus:

1. Prothrombin+Ca+Thromboplastin=Thrombin
2. Fibrinogen+Thrombin=Fibrin

This, the so-called classical theory, has been preeminent up to recent times. This is not to say that other theories have not been put forward; many indeed are the spirited, if sometimes bizarre, heresies that have arisen, so that at times the number of theories has exceeded the number of propounders (Wöhlisch, 1929), but, particularly io the United States and in this country, no attack on this "4-factor" theory has been taken seriously.

Two unorthodox views formulated at the beginning of this century must, however, be considered. The first is that produced by Nolf (1908) aod still vigorously defended by him (1945). In Nolf's view, coagulation requires, in addition to calcium, the interaction of *four* protein-like factors, all of which are present in the plasma. These factors are: thrombokinase (thromboplastin); thrombozyme; thrombogen; and fibrinogen. The thrombokinase of the plasma is a lipoprotein, having the same properties as tissue thrombokinase, and accelerating, in the presence of calcium, the reaction between thrombozyme and thrombogen. These two factors together constitute what was usually called "prothrombin" and which is therefore not a simple substance but a mixture. Neither thrombogen or thrombozyme alone is capable of generating thrombin without the presence of the other, and thrombin is regarded as a product of their union, since its formation coincides with their disappearance. The theory can be illustrated diagrammatically thus:

Thrombozyme
                    Thrombokinase
Thrombogen   ————————————→ Thrombin |→ Fibrin
                                   +
(ca)   Fibrinogen
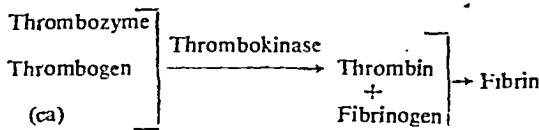
According to Nolf, the thrombozyme-thrombogen complex is responsible not only for the coagulation of fibrinogen but also for fibrinolysis, particularly if the thrombozyme is in excess of its normal ratio to thrombogen. The lifeloog work on blood coagulation of this investigator has received far less attention than it deserves. This is due in part to the rather obscure presentation of his admittedly complex conception and the involved experimental evidence on which it is based. It is largely due, however, to the fact that many modern workers have not troubled to read his published material. As will be seen later, some aspects at least of his theory are receiving unsuspected confirmation.

Another supporter of a "5-factor" theory was Bordet (Bordet and Delange. 1912; Bordet, 1920). He postulated an inert precursor (proserozyme) of what is usually termed prothrombin, which was activated to serozyme by contact with a foreign surface

in the presence of calcium. Serozyme then combined with cytozyme (thromboplastin) to form thrombin, thus:

$$\text{1. Proserozyme} \xrightarrow{\text{contact}+\text{Ca}} \text{Serozyme}$$
$$\text{2. Serozyme}+\text{Cytozyme} \longrightarrow \text{Thrombin}$$
$$\text{3. Thrombin}+\text{Fibrinogen} \longrightarrow \text{Fibrin}$$
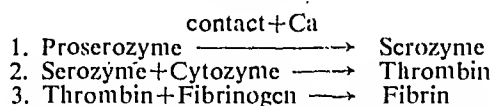
Bordet's theory is based on a number of interesting experiments which may have a significant part in any modern re-orientation of the classical theory, though Bordet's own interpretations may not always be acceptable.

These earlier theories in their preoccupation with clotting did not explain adequately the fluid state of the normal circulating blood. Lister (1863) had emphasized the importance of contact action in the inception of blood coagulation, and it has already been stated that other workers recognized the importance of admixture with tissue fluid containing thromboplastin, or its release following the disruption of cells or platelets when blood is shed. The absence of contact with a foreign surface, however, is not a sufficient explanation of the inherent stability of blood in the circulation, since in a variety of pathological and traumatic conditions both vessels and tissues may be so injured as to permit contact action and the passage of thromboplastin into the blood stream.

It was Howell (1910, 1911, 1935) who first emphasized the possible importance of anticoagulant substances, and who laid the foundations for the idea of an equilibrium between such anticoagulants and the coagulant factors that come most strongly into play when blood is actually shed. He at first postulated an antiprothrombin that was neutralized by thromboplastin. Later he assumed that the newly discovered anticoagulant substance heparin (Howell and Holt, 1918) was identical with this factor. His theory at that time could be illustrated as follows:

1. (Prothrombin – Heparin)+Thromboplastin→
                                        Prothrombin
2. Prothrombin+Ca⟶ Thrombin
3. Thrombin+Fibrinogen⟶ Fibrin
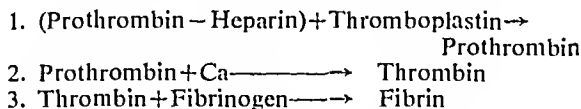
Later work suggests that heparin is mainly an antithrombin, but the concept of anticoagulant factors essential for the preservation of normal blood fluidity still maintains its place in present-day explanations of the coagulation mechanism.

Among workers in this country whose investigations advanced the international knowledge of this subject during the early part of the century, Mellanby (1909) and Pickering (1928) are foremost. The former perfected the technique of separation and study of active fractions of the coagulation mechanism, and by his study of their properties found a solid basis for much modern work (Mellanby, 1930, 1933). Pickering contributed his exhaustive monograph on the subject, together with his synthesis of the divergent and discordant views of his predecessors.

The Factors of the Classical Theory

Before discussing the recent events that have resulted in a modification of the earlier acceptance of the classical theory, some of the well-established facts relating to the familiar components of this system should be reviewed.

Fibrin.—The end-result of normal mammalian blood coagulation is the formation of the fine three-dimensional network of fibres that entangles the formed elements of the blood. Under certain circumstances, principally those involving changes of pH, application of heat, or long storage, clotting produces a structureless gel rather than a reticulum, but this, though of theoretical interest, might be regarded as an artifact.

Examination of the minute structure of the fibrin threads by polariscopic (von Dungern, 1937), radiological (Herman and Worschitz, 1935), or electron-microscopical means (Wolpers and Ruska, 1939; Hawn and Porter, 1947) has suggested that they are composed of needle-like micelles packed lengthwise into bundles. When freshly formed the threads are extremely adhesive, sticking to each other, to the platelets and cells of the blood, to the tissues, and to certain foreign surfaces—a property to which the blood clot owes its haemostatic efficiency.

A curious reaction of freshly coagulated fibrin is that of retraction or, more properly, contraction. The normal blood clot will contract to about 40 per cent of its original volume, squeezing out serum mixed with a small proportion of red cells. Plasma clots will contract to 10 per cent or less of their original volume (Macfarlane, 1939). This activity is reduced naturally in those conditions in which there is thrombocytopenia and can be inhibited artificially by heating to 47° C. or by removing the platelets (Macfarlane, 1938a). The significance of retraction from the point of view of haemostasis is not clear, but there is no doubt that the contracted clot is more solid and elastic than when it is newly formed.

Fibrin is soluble in acids and alkalis, and is digested by proteolytic enzymes. After such solution it cannot be made to re-form into its original reticulum. If it is kept sterile, the fibrin of whole clotted blood will remain intact for days or weeks under normal conditions, but in certain circumstances it undergoes rapid dissolution or fibrinolysis, a process now known to be due to the activation of a proteolytic enzyme of the plasma, and discussed by Macfarlane and Pilling (1946a).

It has been suggested that fibrin may exist temporarily in a soluble form in the immediate preclotting stage. This hypothetical precursor of fibrin has been called profibrin by Apitz (1937), and its

existence is accepted by Astrup (1944) and Owren (1947a). Apitz (1939) believes that a sticky film of profibrin absorbed on to the platelets during coagulation causes their agglutination, an explanation recalling the earlier suggestion of Maltaner and Johnston (1921) that fibrinogen was necessary for the " conglutination " of erythrocytes and bacteria.

Fibrinogen.—Fibrinogen may be described as the fraction of the plasma protein which is coagulable by thrombin. It has the greatest molecular weight of the plasma proteins, a recent estimate being 441,000 (Nanninga, 1946). It is also the most labile, being precipitated by heating to 47° C. or by half saturation with sodium chloride or quarter saturation with ammonium sulphate. Its iso-electric point is pH 5.5 (Nordbö, 1927), and it is precipitated by bringing the pH to this point after reduction of electrolyte by preliminary dialysis or dilution with distilled water.

Wöhlisch and Clamann (1932) and Wöhlisch and Kiesgen (1936) have presented evidence that the molecule of fibrinogen is long and thread-like, this conclusion being in accord with the evidence of the miscellar structure of fibrin already mentioned.

It is generally assumed that fibrinogen is formed by the liver. The evidence for this is as follows: Nolf in 1905 showed that exclusion of the liver from the circulation of dogs resulted in a rapid and profound fall in the blood fibrinogen. This observation was supported by other workers, including Jones and Smith (1930), who observed a similar fall after hepatectomy. Destruction of liver function by the administration of chloroform or phosphorus will also produce the same result (Foster and Whipple, 1921) accompanied, in the case of the former agent, by a decrease in prothrombin as well (Smith, Warner, and Brinkhous, 1937).

In such experiments it is assumed that the fall in fibrinogen is due to the cessation of production by the damaged or excluded liver. the normal rate of fibrinogen utilization being presumably very high. Against this, however, must be set certain objections. The most serious is that substances such as chloroform induce intense fibrinolytic activity, a fact well recognized by workers on this subject. Fibrinolysis was also observed by Macfarlane and Biggs (1946) to occur after any operative procedure, and it has also been shown to occur in severe liver damage. It might be argued, therefore, that the disappearance of fibrinogen in these cases was due to an increased rate of destruction rather than to deficient production (Macfarlane and Biggs, 1948). The second objection involves the normal rate of utilization of fibrinogen. Admit-

tedly in the experimental animals described the rate of utilization may have been higher than normal as a result of haemorrhage. In human beings there is little direct evidence of the normal rate of usage apart from the observation of Pinniger and Prunty (1946) that fibrinogen transfused into a patient with congenital absence of fibrinogen was still present in a detectable amount after ninety-six hours. If this reflects the rate of utilization in normal persons, removal of the source of supply would not have resulted in a detectable drop in the course of the few hours' duration of an acute experiment. The whole question thus requires re-examination.

Most investigators consider that fibrinogen is a homogeneous protein, and that they are able to obtain it in a state of considerable purity by fractionation methods. Some, however, have postulated the existence of different forms of fibrinogen, a view which is now receiving increasing experimental support. As long ago as 1889 Wooldridge regarded fibrin as being derived from the combination of two components, fibrinogen A and fibrinogen B, neither being identical with the fibrinogen separated in the laboratory from plasma, which he regarded as an artifact. Recent work by Nolf (1947) suggests, however, that Wooldridge's fibrinogen A is in some respects similar to the classical thromboplastin, but there are interesting points of difference. Wertheimer and others (1944) have shown that on storage the fibrinogen of plasma separates into a more labile fraction that is precipitated by cooling and a less labile fraction remaining in solution. The proportion of less labile fibrinogen is reduced in liver disease and increased in pregnancy or after haemorrhage.

A very interesting contribution is that of Lyons (1945a. b), who suggests, on the basis of coagulability by naphthoquinone. that there are two forms of fibrinogen—fibrinogen A. which exists in fresh plasma and normal blood, and fibrinogen B (these names are not to be identified with those of Wooldridge), which occurs in certain pathological states and develops on storage of blood and during the clotting of fibrinogen with thrombin. The significance of this work will be discussed later.

Thrombin.—Thrombin is the factor which, though absent from the normal circulating blood. develops during the process of coagulation and on separation can be shown to be capable of clotting fibrinogen. Though it is doubtful if pure thrombin has been obtained or is even obtainable, preparations having very great activity relative to dry weight can be produced with little difficulty. and such preparations can clot several hundred times their own weight of fibrinogen (Eagle, 1935a :

Seegers, 1940 ; Astrup, 1944). Astrup (1944) and his colleagues have shown that thrombin is an albumin-like protein, with a molecular weight of about 75,000. The phosphorus content of Astrup's preparations was too low to be detected by chemical means, a finding that agrees with that of Chargaff and others (1940), who followed the generation of thrombin using a radioactive phosphorus isotope. The calcium content is also negligible (Ferguson, 1937). Thrombin is heat-labile, being destroyed by heating to 60° C., and beginning to lose activity at 40° C.

Lyons (1945b), having found that thrombin digested with trypsin gives a colour reaction characteristic for this substance, maintains that thrombin contains a naphthoquinone group.

Reaction of thrombin with fibrinogen.—The earlier authorities regarded thrombin as an enzyme acting specifically on fibrinogen. Mellanby (1933) called thrombin " thrombase " and thought that it split the fibrinogen molecule into fibrin and a soluble globulin. There was a temporary setback to the enzymatic view, largely due to the work of Howell (1910), from which it appeared that thrombin was consumed quantitatively during the conversion of fibrinogen to fibrin, a fact possibly explained by absorption on to the newly formed fibrin and the presence of antithrombin. Howell's contention was to some extent supported by Hekma (quoted by Pickering, 1928), who thought that thrombin acted as an agglutinin of fibrinogen in the ordinary immunological sense. Pickering (1928) argued against this, pointing out that thrombin is almost inactive at low temperatures, is thermolabile, and soon loses its activity on storage, while agglutinins are more active at low temperatures, are heat stable, and retain their activity for years.

Since more recent work, already quoted, has shown that thrombin can convert at least 600 times its own weight of fibrinogen into fibrin (Astrup, 1944) and that the molecular weight of thrombin is probably not less than one-tenth that of fibrinogen, it seems unlikely that any stochiometric combination of thrombin and fibrinogen can occur, and it seems as if the enzymatic action of thrombin is more probable.

The nature of this enzymatic action is not known. Mellanby's (1933) view that the fibrinogen molecule was split into a soluble and an insoluble fraction is unlikely to be correct, since Jaques (1938) found that the fibrinogen-nitrogen lost during clotting reappeared quantitatively as fibrin-nitrogen. However, the supposition that the process was, in some way, a proteolytic one had some

circumstantial support in that certain proteolytic agents such as papain (Eagle and Harris, 1937) and the proteolytic venoms of *Echis carinata* (Barratt, 1920) and other snakes (Eagle, 1937) can coagulate fibrinogen. Moreover, it was argued that thrombin preparations were often fibrinolytic as well as fibrin-forming, this suggesting that coagulation was merely the prelude to lysis (Nolf, 1938). More recently, however, there is strong evidence to show that the clotting action of thrombin is distinct from any fibrinolytic action which certain preparations may have and which is almost certainly due to contamination with plasmin, the proteolytic enzyme of the plasma (Seegers, 1940 ; Ferguson, 1943 ; Macfarlane and Pilling, 1946a).

In 1941 Baumberger made the fruitful suggestion that thrombin contributed to the formation of fibrin by potentiating a chemical linkage between fibrinogen molecules. He considered that the SH groups known to be present in the fibrinogen molecule might be oxidized by thrombin, so that an S-S linkage between fibrinogen molecules could occur, with consequent construction of the fibrin lattice, thus :

$$2 \text{ (Fibrinogen—SH)} \xrightarrow{\text{Thrombin}} \text{Fibrinogen—S-S—Fibrinogen}$$
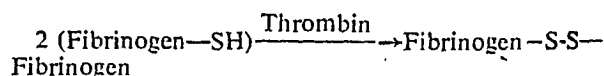
This conception has been extended by Lyons (1945a and b). It has already been stated that he believes that the thrombin molecule contains a naphthoquinone group, and that this substance is able, by itself, to clot a certain form of fibrinogen known as fibrinogen B. He explains the previous observations that ninhydrin will clot fibrinogen (Chargaff and Ziff, 1941) on the basis of the similarity of the structure of this substance to the naphthoquinones (Fig. 2). The failure of others to

3, 3′-Methylene-bis-(4-hydroxycoumarin)

2-Methyl-1,4-naphthoquinone

Ninhydrin
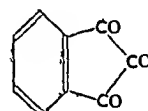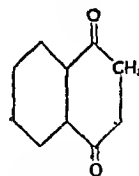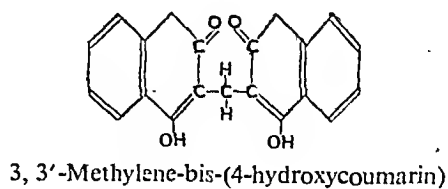
FIG. 2.—Structural formulae of certain substances active in the coagulation mechanism.

obtain coagulation of fibrinogen by naphthoquinone is due to the use of fibrinogen A in their experiments. Lyons suggests that in its native form fibrinogen has its thiol groups blocked, this form constituting fibrinogen A. On storage, or in certain pathological states, fibrinogen A may become partly converted to fibrinogen B, in which the SH groups are free. Such fibrinogen is coagulable by ninhydrin and the naphthoquinones. During natural coagulation fibrinogen A is acted upon by a component of thrombin (thrombin A), the thiol groups being freed. The fibrinogen B thus formed is then clotted by the naphthoquinone component of thrombin (thrombin B) thus:

1. Fibrinogen−SH (blocked)+Thrombin A
   (Fibrinogen A) ↓
2. Fibrinogen−SH+Thrombin B (or naphthoquinone)
   (Fibrinogen B) ↓
3. Fibrinogen−S-S−Fibrinogen (Fibrin gel)

This work, which is supported by a number of points of evidence, is of considerable interest and, if confirmed, of obvious practical and academic importance.

Laki and Mommaerts (1945) have also postulated a two-stage reaction in the conversion of fibrinogen to fibrin, but have been less precise as to the mechanism.

Prothrombin.—Prothrombin is the inactive precursor of thrombin, and, as such, is present in the circulating blood. It has the physical and chemical attributes of a globulin (Astrup, 1944), being precipitated at pH of 5.6 and by half saturation with ammonium sulphate. Like thrombin its activity is destroyed by heating to 60° C. It is strongly adsorbed by such substances as $BaSO_4$ (Dale and Walpole, 1916), $Ca_3(PO_4)_2$ (Bordet and Delange, 1914), $Mg(OH)_2$ (Fuchs, 1929), and $Al(OH)_3$ (Quick, 1935a). It is also adsorbed by Seitz or Berkfeld filters. It is destroyed by proteolytic enzymes (Eagle and Harris, 1937).

The evidence that prothrombin is produced by the liver is similar to that purporting to show that fibrinogen has the same origin, that is, upon the observation of prothrombin deficiency arising after chloroform poisoning (Smith and others, 1937) or partial hepatectomy (Warner, 1938). Since prothrombin is destroyed by proteolytic enzymes and the experimental procedures described are potent activators of the proteolytic system of the blood, the same objections to these conclusions can be made as were discussed in relation to the site of origin of fibrinogen.

Vitamin K.—The story of the discovery of vitamin K and of its essential part in prothrombin production by the body now forms a familiar page in the history of fundamental medical advancement. It is instructive and stimulating to study the rapid unravelling of threads leading to an understanding of a whole series of haemorrhagic disorders that resulted from the brilliant and often independent contributions produced in quick succession. In 1934 Dam and Schönheyder reinvestigated the haemorrhagic disease that develops in chicks fed on a synthetic diet, and Dam (1935) decided that it was due to the absence of a factor (coagulation-vitamin or vitamin " K ") required for the proper clotting of the blood. In the same year Halbrook (quoted by Quick, 1942) showed that the condition was cured by the administration of alfalfa (lucerne) or its ether extract, a finding confirmed and expanded by Almquist and Stokstad (1935). Further investigations by Dam and others (1936) showed that the haemorrhagic disease was due to a prothrombin deficiency, a conclusion reached independently by Quick (1937), who also showed that the prothrombin level could be restored to normal by feeding alfalfa. Quick (1935b) had already discovered the prothrombin deficiency in obstructive jaundice and he (1937) was not slow to predict that this might be due to a deficiency of the new (fat-soluble) vitamin because of deficient adsorption in the absence of bile salts from the intestine. He was able to substantiate this prediction (1938a) by actual clinical observations, which were confirmed by Warner and others (1939a) and by Butt and others (1938).

Efforts were now made to isolate the new substance. It was found that it is widely distributed in plants, particularly in green leaves (Dam and Glavind, 1938), and that bacteria can synthetize the vitamin, so that its production in the animal intestine may be an important factor in its natural assimilation (Almquist and others, 1938). Scientific methods of assay (Almquist and Klose, 1939a) based on Quick's (1935b) prothrombin-time method greatly assisted this progress in the study of the distribution and synthesis of vitamin K. As regards the chemistry of vitamin K, the principal work was carried out by Dam and his co-workers on the one hand and Almquist's group on the other (see review by Almquist, 1941). The structure of two naturally occurring substances with vitamin-K activity were quickly determined, both being derivatives of 1,4-naphthoquinone. Almquist and Klose (1939b) found that phthiocol, a compound isolated from tubercle bacilli, had vitamin-K activity and was also a 1,4-naphthoquinone derivative. It was then almost simultaneously discovered by four separate groups of workers (Almquist and

Klose, 1939b; Ansbacher and Fernholz, 1939; Fieser and others, 1939; Thayer and others, 1939) that the comparatively simple synthetic substance 2-methyl-1,4-naphthoquinone had even greater vitamin-K activity than the naturally occurring compounds. Since that time a number of derivatives of this substance with the added advantage of solubility in water have been produced commercially under different trade names, and vitamin K has taken its place among the factors essential for life.

Its mode of action in the production of prothrombin is at present unknown, though the observations of Lyons (1945b) suggest that it is an integral part of the thrombin molecule. It has no effect on prothrombin activity *in vitro* and its administration in cases of hypoprothrombinaemia is effective only if the function of the liver is relatively normal. In the latter case the rise in prothrombin following vitamin-K administration is very rapid, a tenfold increase occurring within twelve hours in some patients (Quick, 1942).

Calcium.—It is now undisputed that ionized calcium is required for the conversion of prothrombin to thrombin, but that the latter can act on fibrinogen with unimpaired effect in· the absence of calcium or in the presence of decalcifying agents such as oxalate or citrate.

The calcium of the blood-plasma is present in two forms; the diffusible ionized calcium and the non-diffusible protein-bound fraction, which is usually thought not to take·part in the coagulation process. Removal of free calcium by precipitating it as the oxalate, or suppression of ionization by the addition of citrate, will inhibit the normal clotting process indefinitely. It is significant, however, that if clotting is to be prevented at least three times as much oxalate must be added to plasma as the calculated amount necessary to precipitate all the available calcium (Vines, 1921; Scott and Chamberlain, 1934; Quick 1940). This finding is difficult to understand unless it is supposed that there is a competition for available calcium by the coagulation factors on the one hand and the decalcifying agent on the other, it being necessary to have an excess of the latter to deprive the former of calcium by mass action.

Ferguson (1937) supposed that an intermediate prothrombin-calcium-thromboplastin complex was formed during clotting and that this complex could be inactivated by decalcification. Quick (1940) also postulates a prothrombin-calcium complex, from which calcium has to be removed before the decalcifying agent can prevent coagulation. He has also provided evidence (1947b) that there is a calcium co-factor associated with, but distinct from, prothrombin. Greville and Lehman (1944) have studied the interesting cation antagonism affecting this part of the clotting mechanism, in which certain ions, particularly those of magnesium, antagonize the effect of calcium.

The amount of calcium required for effective coagulation has a wide optimum value, ranging from 0.025 to 0.0025 molar concentration. Above and below this level coagulation is depressed and finally inhibited (Quick, 1942).

Thromboplastin.—Although blood which is collected without contamination with tissue fluid and which is then kept in a container with a "neutral" (non-water-wettable) surface will not clot, it can be made to do so with great rapidity by the addition of a small quantity of the watery extract of almost any tissue. It can therefore be assumed that prothrombin, even in the presence of calcium, is inactive, but that it can be activated by a factor or complex of factors present in the tissues. This agent has received various names, such as cytozyme, thrombokinase, thromboplastin, zymoplastic substance, or simply kinase. It occurs in most tissues, but brain, lung, thymus, testes, and platelets yield the most active preparations, and it can be demonstrated in human saliva (Bellis and Scott, 1932).

In discussing the nature, properties, and modes of action of thromboplastin a major difficulty arises which is by no means limited to this particular aspect of the coagulation problem. This is the probability that "thromboplastin" has more than one component, and that different substances, claimed by various workers to be thromboplastin in a state of relative purity, are in fact different fractions of the whole.

The original zymoplastic substance used by Schmidt (1895) and his associates was an alcohol extract of tissue, and was thought to contain lecithin as the active factor. Morawitz (1905) showed that aqueous tissue solutions had the same effect and were, moreover, heat-labile; he concluded that the active factor was a protein, and called it "thrombokinase." Howell (1912) showed that both extraction by water or by lipoid solvents yielded active material, and he applied the now-popular term "thromboplastin" to the water-extractible fraction. He was able to show that the ether-soluble fraction was cephalin, which itself was active as a thromboplastin, though, when in combination with an unknown protein factor, the activity was greatly increased. The cephalin was heat-stable as regards activity, but the cephalin-protein complex was heat-labile. Confirmation for

the suggestion that cephalin was an active component of thromboplastin came from a number of investigators, including Gratia and Levene (1922), McLean (1916), and Quick (1936a), while lecithin was shown to be inactive (Clowes, 1917; Gratia and Levene, 1922). A particularly active cephalin preparation has been isolated from platelets by Chargaff and others (1936). Cohen and Chargaff (1940) supported Howell's belief that thrombokinase was a lipoprotein and suggested that the protein component was effective in orientating the cephalin at its surface. Chargaff and others (1944) have studied the chemistry of a lung preparation that is homogeneous in the ultra-centrifuge. They could not separate an active protein component. The lung preparations are more stable to heat than the brain extracts, and Lenggenhager (1936) has shown that even boiling does not greatly impair their activity. Quick (1942) has made similar observations.

Further indications that thromboplastin is a complex factor can be quoted. Though prothrombin can be activated by calcium and pure cephalin, the process is very slow compared with the reaction when crude tissue preparations are used (Seegers and others, 1938). A potent thromboplastin has been prepared from lung that contains no significant amount of cephalin, a finding of obvious importance (Charles and others, 1934).

It is possible that the action of Russell's viper venom may eventually throw some light on this problem. The fact that certain snake venoms were potent coagulants of blood was recognized by Martin in 1894, and their action was investigated by Lamb (1903) and Mellanby (1909). The prevalent use of oxalated or citrated blood as an indicator of activity, however, obscured the extreme potency of the venom of Russell's viper, which was pointed out by Macfarlane and Barnet (1934) in their search for a local haemostatic agent that might be effective in haemophilia. They found that haemophilic blood was rapidly clotted by extremely small amounts of venom, a dilution of one in many million still having a marked effect. The venom was incapable of clotting oxalated or citrated blood and had no effect on fibrinogen. It behaved in fact as if it were a thromboplastin.

Further work by Trevan and Macfarlane (1936) demonstrated the fact that the action of the venom was greatly increased by the presence of tissue lipoids, or lecithin, suggesting that the latter acted as a co-factor with the venom in its thromboplastic role. Macfarlane (1937, unpublished observations) found that the venom alone was unable to clot plasma from which the lipoid had been removed by extraction with petroleum ether, but that rapid coagulation occurred if the extracted lipoid or lecithin were mixed with the venom before addition to the plasma. He also observed that the coagulant extractable from human saliva behaved in the same way, since it would only clot the extracted plasma in the presence of added lipoid. These experiments extended the observations of Zak (1912) that plasma extracted with petroleum ether will not clot on recalcification unless the lipoid fraction is returned. Macfarlane and others (1941) obtained similar results with samples of plasma from which lipoid had been removed by high-speed centrifugalization, and suggested, as had Leathes and Mellanby (1939), that the venom was analogous to an enzyme (protein) component of natural thromboplastin, the lecithin being analogous to the natural lipoid co-factor.

The complex nature of thromboplastin has been emphasized by Feissly (1945a), who has extracted a thermostable phospholipoid from platelets and, a thermolabile proteolipoid from plasma. Lenggenhager (1946) also considers that more than one factor is involved, since he supposes that prothrombokinin, a precursor of thrombokinin (? thromboplastin), is activated by proteolysis.

The findings of Zondek and others (1945) that thromboplastic substances derived from placental tissue were heat-stable when fresh but heat-labile after some days' storage emphasizes the difficulty of interpretation of published observations. Nevertheless it is clear that further experiments along the lines indicated may clarify the problem of the nature of thromboplastin, and recent work with haemophilic blood is already showing promising results.

Thrombin formation.—The nature of the reaction between thromboplastin, calcium, and prothrombin has given rise to endless discussion, fostered by uncertainty as to the " purity " of the various investigators' preparations of these factors, and the possibility that other undefined agents were involved.

The original view of Bordet and Delange (1912), supported in more modern times by Lenggenhager (1935), was that the three factors combined chemically to form a new compound, which was thrombin. It now seems most unlikely that this can be the case. It has already been said that the thrombin molecule appears to be smaller than the prothrombin from which it is derived, so that any simple combination of thromboplastin and prothrombin is, on the face of it, impossible. Moreover, it has been shown that thrombin contains no

demonstrable phosphorus (a considerable com-
ponent of thromboplastin), and no calcium.

It would seem most likely, therefore, that
thrombokinase activates prothrombin by removing
some part of its molecule. Howell (1935) con-
tended that this process consisted of the removal
of heparin, which normally formed an inert
compound with prothrombin. Quick (1936b),
however, was not able to demonstrate the neutral-
ization of heparin by thromboplastin, and, in any
case, heparin exerts its main anticoagulant effect
on the action of thrombin. There is, on the other
hand, some evidence to favour the idea that one
component of thromboplastin is an enzyme. The
objection that thromboplastin has been stated to
be heat-stable may well apply only to the lipoid
co-factor which has been mistaken for the whole
complex.

The significance of the activity of Russell's
viper venom, which is almost certainly enzymatic,
and its co-factor lecithin, has already been
mentioned. There is additional support for pro-
teolysis in the action of trypsin. Douglas and
Colebrook (1916) observed that trypsin hastened
the clotting of blood, a finding confirmed by
Heard (1917), who found, moreover, that the
enzyme was capable of coagulating oxalated
blood. Mellanby (1935) and Mellanby and Pratt
(1938), investigating this finding, came to the con-
clusion that trypsin activated the thromboplastin
of the blood, and that the calcium present in the
trypsin afforded sufficient to allow clotting. Eagle
and Harris (1937), however, obtained the same
result with calcium-free crystalline trypsin, and
concluded that the prothrombin molecule was split
proteolytically to form thrombin. Ferguson and
Erickson (1939) concluded that the action of
trypsin was to free a cephalin-calcium complex.
It is of interest that a number of proteolytic snake
venoms have a coagulant action similar to trypsin
(Eagle, 1937).   ～

It may be significant that substances with an
anti-proteolytic effect have also been shown to be
anticoagulant. The pancreatic trypsin inhibitor
of Kunitz and Northrop (1936) is strongly anti-
coagulant (Ferguson, 1942), and the soya bean
trypsin inhibitor isolated by Kunitz (1945) has
been shown by Macfarlane and Pilling (1946b) and
Macfarlane (1947) to act as an antithromboplastin.

The evidence seems, therefore, to favour the
existence of a thromboplastic enzyme (kinase)
which, in the presence of a lipoid activator and
calcium, splits the prothrombin molecule. This
enzyme is present with its activator in most tissues,
and extracts of such tissues are spontaneously

active. It is also probable that the enzyme exists
in the plasma as an inert precursor which is acti-
vated by contact with a foreign surface or by some
other factor resulting from such contact. This
precursor is possibly identical with Lenggenhager's
" proplasmakinin," Feissly's (1945b) " plasma
thrombokinase," Macfarlane's (1945) " prokinase,"
and the so-called " antihaemophilic globulin " first
described by Patek and Taylor (1937). Since the
systems used by most investigators to test thrombo-
plastic activity may be deficient in lipoid co-
factor, but contain unsuspected and significant
amounts of prokinase, it follows that the addition
of lipoid may produce an acceleration of coagula-
tion which may be taken to indicate the thrombo-
plastic activity of the added lipoid.

An argument against the enzymatic, possibly
proteolytic, activation of prothrombin is the
apparent consumption of thrombokinase during
thrombin formation. Though Eagle (1935a) con-
sidered that the amount of thrombin generated
in a mixture of prothrombin, calcium, and
thrombokinase was independent of the amount
of thromboplastin, the work of Mertz and others
(1939) suggests that, for low concentrations, a
given amount of thromboplastin causes the con-
version of a given amount of prothrombin, a
finding taken to disprove the enzymatic nature of
the reaction. Such an observation, however,
might merely mean that it is the lipoid factor that
is consumed during the process of activation.

The Inception of Blood Clotting

For many years the search for the prime mover
of the coagulation mechanism has been under-
taken without very convincing results. It has been
recognized for more than a century that the intro-
duction of tissue emulsions or extracts into the
blood stream will result in intravascular coagula-
tion in the living subject (de Blainville, 1834;
Wooldridge, 1886). The coagulation of blood
escaping from the body and mixed with the pro-
ducts of damaged tissue would seem, therefore, to
be due to this admixture, and it has already been
shown that such tissue fluids contain active
thrombokinase. It can be argued, therefore, that
active thromboplastin is capable of clotting blood
without the intervention of any other agency; yet
it is a familiar observation that blood removed
with the greatest care to avoid admixture with
tissue juice still clots in a short time on contact
with glass or other foreign surfaces. The larger
the surface area with which it is in contact, the
more rapid the clotting, hence the coagulant effect
of substances like cotton-wool or bandages and

their practical use in haemostasis, and the fact that the coagulation time of blood samples is faster in small tubes than large ones.

Lister (1863) was one of the first to point out the importance of such contact and showed that certain surfaces such as rubber delayed blood clotting, as compared with others such as glass. It soon became established that certain neutral substances such as paraffin (Freund, 1888), collodion, lusteroid (Lozner and others, 1942), and most recently silicone (Jaques and others, 1946), which are water-repellent and are not wetted by blood, have very little stimulating effect on coagulation, so that blood collected without tissue fluid contamination will remain fluid for long periods in vessels made of, or coated with, these materials.

The most familiar explanation of these observations is that originally put forward by Morawitz (1905), who supposed that active thrombokinase was liberated by the platelets which rapidly disintegrated when blood was shed. Calcium is necessary for such disintegration (Cramer and Pringle, 1912), and it has been shown by Tait and Green (1926) and Lampert (1930) that the surfaces active in promoting coagulation are those which cause disintegration of platelets.

In favour of this hypothesis it has been established that platelets contain thromboplastin, and significant changes in their morphology have been observed by Nygaard (1941) at the beginning of the clotting process.

It seems, however, that this is not the whole explanation. It is known, for instance, that the clotting time of whole blood as measured by the method of Lee and White (1913) is considerably influenced by small changes in thromboplastin (Quick, 1942), yet in severe thrombocytopenic purpura, in which the platelets may be almost entirely absent from the blood, a significant increase in the clotting time of the whole blood does not usually occur. An important finding of Lozner and others (1942) is that the effect of foreign surfaces on blood coagulation is largely independent of the platelets, there being acceleration or delay in glass or lusteroid respectively of the clotting time of plasma previously deprived of platelets by centrifuging. These authors, therefore, favour the view, already put forward by a number of others from Mellanby (1909) onwards, that there exists an inactive precursor of thrombokinase in the plasma and that this is activated in some way by contact with a foreign surface.

Recent observations by Brinkhous (1947) and Quick (1947b), in relation to the clotting effect in haemophilia, may have an important bearing on the normal first stage of coagulation. With the use of silicone, plasma can be prepared free of platelets and without surface contact action. On transfer to a glass surface such plasma will not clot without the addition of platelets. Brinkhous (1947) infers the existence of a "thrombocytolysin" that disrupts the platelets on contact with a foreign surface with release of thromboplastin. Quick (1947b) postulates a factor released by platelets on contact that activates "thromboplastinogen." It might equally be argued, from what has already been said, that platelets on rupture supply the "lipoid factor" which reacts with the contact-activated "prokinase" or thrombokinase precursor.

The nature of such contact action is almost certainly physical. As Pickering (1928) writes, "Electrical changes produced by contact of blood with a surface which it wets seems sufficient for the inauguration of clotting. A wetted surface becomes electro-negative and the presence of a negative charge implies an equal positive charge in the liquid in contact with it . . . a change of electrical conditions is thus a feature of normal clotting." What other factors intervene between such contact and the activation of prokinase (or thromboplastinogen) are not known. It has already been stated that Lenggenhager considers that proteolysis plays a part, a view favoured in principle by Wöhlisch (1940) and Ferguson and others (1947). If this is so, the whole fibrinolytic system, which results in the activation of the proteolytic enzyme plasmin (see Macfarlane and Biggs, 1948), may be concerned with the inception of blood clotting.

The Maintenance of Normal Blood Fluidity

Many workers on the subject of blood coagulation have been so preoccupied with an explanation of the change from fluid to solid that their theories hardly explain why the blood is fluid in the body. Yet it is clearly as important to the individual that his blood should not clot in his vessels as that it should clot outside them.

Normally the blood has an inherent stability in the body. The main reason why intravascular coagulation does not occur is the unbroken continuity of the vascular endothelial surface, which is inactive as regards clotting. Nevertheless, it is not only on this absence of foreign contact that the body relies. Disease may alter the vascular endothelium, but no more than a local deposit of platelets and fibrin may form. Trauma may cause extensive damage to tissues and vessels, with inevitable absorption of thromboplastin and

thrombin, but only local thrombosis of the affected vessels occurs in the majority of cases. Though intravascular clotting can, as has been said, be produced by the injection of tissue extracts, relatively large amounts have to be given to produce it, and even thrombin given intravenously may have no apparent effect (Davis, 1911).

The classical theory does not explain this reluctance of the mass of circulating blood to clot in the presence of active coagulants. It is almost certainly due to the presence of anticoagulant factors, which, though demonstrable, have received comparatively little attention and which probably maintain a dynamic equilibrium with coagulant factors that are slowly produced in even normal circulating blood.

It was early recognized that thrombin rapidly disappeared from serum after coagulation was complete. Schmidt (1892), in studying this problem, paid particular attention to the fact that after such spontaneous inactivation the thrombin could be regenerated by the addition of acid or alkali. Morawitz (1924) came to the conclusion that thrombin was converted into an inactive form which he named " metathrombin," and Weymouth (1913) and Gasser (1917) concluded that there is a substance, or antithrombin, présent in serum with which thrombin combines and by which it is therefore inactivated. No further progress was made until 1935, when Lenggenhager stated that this antithrombic factor was associated with the albumin fraction of the serum, a finding confirmed by Quick (1938b), who later called it " albumin X " (Quick, 1942) and showed that it had a lesser affinity for thrombin than had fibrinogen. Glazko and Ferguson (1940) have called it the " progressive antithrombin " to distinguish it from heparin and its co-factor, which they called the " immediate antithrombin." It has been studied by Astrup (1944), who calls it simply " antithrombin." Wilson (1944) believes that the thrombin is adsorbed directly by the albumin, but Wöhlisch and Köhler (1942) and Grüning (1943) find that the antithrombin activity of serum albumin is removed by extraction with chloroform or ether. The ether extract is itself antithrombic and appears to be a lipoid. Antithrombin has, therefore, similarities to the serum antitrypsin, or antiplasmin, which is also a labile factor associated with serum albumin and extractable with lipoid solvents (see Macfarlane and Biggs, 1948), and it is of interest that proteolytic enzymes such as trypsin are capable of regenerating from a thrombin–antithrombin mixture (Wöhlisch and Köhler, 1942).

An anticoagulant which has been more intensively investigated is heparin. Discovered by McLean (1916), it was thought by Howell (Howell and Holt, 1918) to be the antiprothrombin required by his theory. Mellanby (1934) and Quick (1936b), however, were able to show that the main action of heparin was against thrombin and not prothrombin or thromboplastin, though Brinkhous (1939) later showed that there was some inhibition of prothrombin conversion in the presence of heparin.

The action of heparin on thrombin, however, is not direct. Howell and Holt (1918) showed that it had little effect on thrombin and fibrinogen, and Quick (1936b) showed that this was due to the absence of a co-factor present in the albumin fraction of whole plasma without which heparin loses its effect. This co-factor has also been studied by Astrup (1944), who finds that it is more labile than the serum antithrombin, being destroyed by heating to 56° C., and that it disappears during the process of coagulation. The heparin co-factor complex has been called " immediate antithrombin " by Glazko and Ferguson (1940), and " thrombin inhibitor " by Astrup (1944), the co-factor being called " thrombin co-inhibitor."

Heparin was purified by Charles and Scott in 1933 and found to occur in liver, lung, muscle, heart, and blood. The chemistry of the substance was studied by Jorpes and Bergström (1937), who established the fact that it is a mucoitin polysulphuric acid ester. Hölmgren and Wilander (1937) showed that the basophil granules of tissue mast cells are probably composed of heparin, a supposition recently confirmed by Oliver and others (1947).

It is likely (Astrup, 1944) that heparin exerts its effect by virtue of the strongly charged sulphuric acid group. Bergström (1936) has synthetized sulphuric acid esters of different polysaccharides, and some, including those of chitin, cellulose, and pectin acid, have an anticoagulant action like that of heparin. It is significant that the anticoagulant azo dyes also contain a sulphuric acid group.

Jaques and others (1942) have shown that the heparins isolated from different animal species have different anticoagulant activity.

The importance of heparin as a physiological anticoagulant is still uncertain. If it is present in normal blood, it is in small amounts, but certain conditions as, for example, anaphylactic shock (Howell, 1925 ; Jaques and Waters, 1941) or heavy exposure to irradiation (Allen and Jacobson, 1947)

may cause such a rise in blood heparin that partial or complete incoagulability results.

Other anticoagulant agents in the normal clotting mechanism, though postulated, have not been definitely established. Collingwood and MacMahon (1912) have suggested an antithromboplastin, and Tocantins (1944a, b ; 1945) has produced evidence that such a factor may exist, being, he thinks, increased in amount in haemophilia.

Quantitative Studies on the Coagulation Factors

It is not until the study of a particular reaction has reached the stage at which the factors concerned can be measured quantitatively that it can be termed a scientific investigation. Though certain of the more stable clotting factors have been the subjects of quantitative study, it is only in the past few years that the main developments along this line have taken place. The stimulus has undoubtedly been the discovery of vitamin K and the condition of hypoprothrombinaemia, both being dependent on the determination of prothrombin concentration ; and the use of anticoagulants in the control of thrombosis must itself be controlled if the treatment is not to be more dangerous than the disease. It is obvious that a general change in attitude towards the problem of coagulation has sprung from these necessities, and its study from being a pleasant pastime for argumentative academicians has grown up overnight into a practical science with vital applications.

The effect of the increased precision of thought and technique which this new development has demanded has not, however, been to simplify the general picture of the coagulation process. On the contrary, it has been found that new factors and new and complicated interrelationships must be postulated if the new quantitative results are to be intelligible. These have, for the moment, been wedged into the already rather rickety framework of the "classical theory." Whether it will continue to bear this increased weight, or whether it will collapse to be replaced by a new structure, remains to be seen. These forebodings can be illustrated best by considering the various quantitative procedures and the complications that have arisen from them.

Simple blood coagulation time.—The time taken for blood to clot after its removal from the body provides the oldest and simplest of the quantitative approaches to the problem of coagulation and was estimated by most of the early workers on the subject. Many of them devised their own methods, some of considerable ingenuity, and it would be impossible to embark on even a brief survey of the instruments

constructed, varying in complexity as they do from an inch or so of capillary glass tubing to a room full of electrical machinery.

In general the simplest methods are the best for ordinary purposes, and the methods in common use to-day require no special apparatus. Blood obtained by venepuncture is preferable to that from a skin puncture, because in the former case, assuming reasonable technical skill, there is a minimum admixture with tissue juice, which in capillary blood may be present in variable amounts, thus adding an uncontrollable variable to the estimation. For venous blood samples the method of Lee and White (1913) is usually employed. In patients in whom venepuncture is contraindicated capillary blood samples are usually tested by the methods of Dale and Laidlaw (1911), Wright (1893), Sabrazes (1904), or Bürker (1907).

Prolongation of the simple coagulation time gives evidence of gross impairment of the coagulability of the blood, such as is met with in haemophilia, fibrinopenia, or severe hypoprothrombinaemia. It does not, however, give a significant indication of the level of fibrinogen until this is almost completely absent (Schmitz, 1933), prothrombin until it is less than 10 per cent of the normal value (Quick, 1945a), and calcium unless this is below the level at which tetany usually occurs (personal observation). In the majority of cases the main factor controlling the clotting time of whole blood seems, therefore, to be the concentration of thromboplastin (Quick, 1942). All other factors are normally present in considerable excess for the production of fibrin within the time of this estimation.

As such estimations are commonly performed there are a number of factors which may influence the results. Small amounts of tissue juice may greatly shorten the clotting time of haemophilic blood. Dirty tubes or syringes may introduce significant variations. Temperature must be controlled carefully, since it has a considerable effect. Fig. 3 illustrates the effect of varying temperature on the clotting time of normal blood in a mechanical coagulometer of the author's design (Macfarlane, 1938a). At the temperatures above 47° C. it was observed that clotting was poor, part of the fibrinogen being precipitated before coagulation could occur.

Fibrinogen and calcium.—The two substances which have been susceptible to ordinary biochemical assay methods are calcium and fibrinogen. The former has not involved any serious difficulty from the point of view of coagulation theory, though its exact part in the mechanism is still not known.

Fibrinogen.—The estimation of fibrinogen usually involves the clotting of a given amount of oxalated plasma by recalcification, removal of the fibrin, washing it free of soluble protein, and the determination of its mass by either drying and weighing or by nitrogen determination. Apart from technical errors and difficulties, this method assumes that all the available fibrinogen is converted into fibrin by this process, and
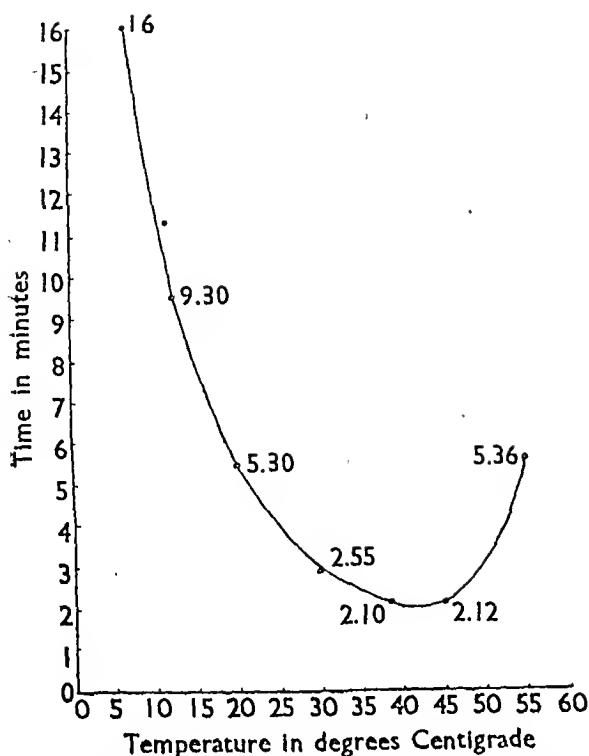
FIG. 3.—Curve showing the effect of temperature on the coagulation time of normal whole blood. (Macfarlane, 1938a.)

0.1 ml. of each dilution to 0.2 ml. of normal oxalated plasma at 37° C., taking the time of coagulation for each dilution. From the curve of clotting times plotted against dilutions, the strength of an unknown thrombin is read off by estimating the coagulation time with the same plasma. Such a method, of course, merely gives the value of the unknown in terms of the arbitary standard, and, as has been shown by Owren (1947a) and Astrup (1944), oxalated plasma is apt to give variable results even with the same thrombin dilutions. These difficulties are almost certainly due, in part, to the presence of antithrombin which will affect the longer clotting times of weak thrombin dilutions more than the shorter times (Fig. 4).

The use of fibrinogen solutions, prepared by removing prothrombin by adsorption with magnesium hydroxide and subsequent salt precipitation, allowed Warner and others (1936) to define a " thrombin unit " as that amount of thrombin that would clot 1 ml. of 0.08→0.1 per cent fibrinogen in 15 seconds at 28° C. There are, however, difficulties even with this refinement of the method. The reaction time is considerably influenced by such variables as pH, protein and salt concentration, and osmotic pressure (Quick, 1942 ; Owren, 1947a), and Astrup (1944) was forced to use a dried preparation of thrombin as a reference standard in his precise quantitative investigations. Some of these difficulties have been explained by Owren (1947a), who has shown that fibrinogen solutions prepared in the ordinary way

that one milligramme of fibrin is derived from one milligramme of fibrinogen. The latter assumption is probably correct, but the former is not necessarily so. In any condition in which thrombin production is defective only a part of the fibrinogen may be converted, and subsequent addition of thrombin may produce another clot. It is also doubtful if even added thrombin removes all the protein which should strictly be regarded as fibrinogen. It has been found in the writer's laboratory that after a given amount of fibrinogen has been clotted by thrombin there is still 10 per cent left in solution that can be precipitated by heating to 47° C. or by quarter-saturation with ammonium sulphate.

The estimation of thrombin activity.—The estimation of the activity of a given solution of thrombin is the essential basis of quantitative assay of any of the other coagulant factors, since, of all the reactions involved in clotting it is only the reaction between thrombin and fibrinogen that gives a visible effect. In principle, the thrombin to be tested is added to either oxalated plasma (Mellanby, 1933) or fibrinogen solution (Warner and others, 1936), and the clotting time determined. Mellanby (1933) defined a thrombin unit as that amount required to clot 1 ml. of oxalated plasma in 30 seconds at 37° C. For actual assay, Quick (1942) prepares a " standard thrombin " from a given volume of normal plasma, makes serial dilutions of this from full strength to 1/300, and adds

FIG. 4.—Curves showing the relationship of thrombin concentration to clotting time of oxalated plasma (*A*) and fibrinogen solution (*B*). (After Owren, 1947a.)

contain variable amounts of profibrin, which react more rapidly with thrombin than ordinary fibrinogen. Profibrin-free fibrinogen, which is therefore essential for accurate work, can be prepared by centrifuging off the bulk of the profibrin in the cold from freshly thawed frozen fibrinogen, and adsorbing the rest with kaolin. The resulting clear solutions of fibrinogen give regular results.

The estimation of thromboplastin.—The estimation of thromboplastic activity is one of great technical difficulty, since it requires adequate standardization and control of all the other known (and unknown) factors involved in the coagulation process. Fischer (1935a) used bird plasma as an indication of thrombokinase activity, but in view of the known species specificity of the clotting factors (Quick, 1942) such a system may not be applicable to mammalian thromboplastin. Mills (1921) deduced a formula for the assay of thromboplastin which has been also used by Astrup and Darling (1942). Astrup (1944), however, has found that there are differences in the qualitative reactivity of thromboplastins prepared from different organs which introduced uncertainty, brain thromboplastin being less reliable than lung preparations. Other fallacies have been discussed by Owren (1947a), who illustrates the inhibitory effect on coagulation of excessive concentrations of thromboplastin. Quick (1936a) and Aggeler and Lucia (1938) have used oxalated plasma for assay of thromboplastin.

The estimation of prothrombin.—The development of practical methods for the estimation of prothrombin was essential to the most fruitful research in blood coagulation, the discovery of vitamin K, the hypoprothrombinaemic states, and dicoumarin.

Quick (1935b), during an investigation of the haemorrhagic tendency in jaundice, developed his method of prothrombin estimation which has proved so useful. In principle, it depends on the assumption that there are four reactants in the coagulation mechanism—thromboplastin, prothrombin, calcium, and fibrinogen. If any three of these are fixed, variations in the clotting time of the mixture must, it is assumed, be due to variations in the fourth factor. To determine the prothrombin concentration the concentration of thrombokinase, calcium, and fibrinogen should be controlled, preferably at their optimum concentration for coagulation, so that the clotting time of such a system should then be proportional to the concentration of prothrombin. In practice it is found (Quick, 1942) that fibrinogen variations in human plasma are not usually of such magnitude as to introduce a significant error, and the calcium concentration can be controlled by using oxalated plasma and a known amount of calcium chloride.

The remaining variable to be controlled is thromboplastin. In ordinary whole blood this is present in amounts so far below the optimum that variations in other factors have little effect on the clotting time. Quick therefore added "excess" thromboplastin—in the form of rabbit brain emulsion (later changed to acetone-extracted dried rabbit brain made up in saline (Quick, 1942)—to the oxalated plasma to be tested, together with the optimum amount of calcium chloride, and recorded the coagulation time. At first the results of this estimation were expressed in terms of the clotting time of the mixture, a normal control plasma being used. In 1938, however, Quick published a curve relating the clotted time by this method to the prothrombin concentration. This curve was constructed by making a number of dilutions of normal and abnormal plasma samples with saline, and from it percentage of prothrombin can be read off, assuming that 100 per cent is an average normal content, and that the thromboplastin used gives the clotting time with normal plasma that is equivalent to 100 per cent on the curve (Fig. 5). The theoretical basis of this curve is, of course, open to several objections. In the first place, as pointed out by Aggeler and others (1946) it was not constructed from a sufficiently large number of normal individuals, and the 100 per cent figure is quite arbitrary. More serious is the assumption that plasma diluted with saline is equivalent to undiluted plasma with a reduced prothrombin content. It is clear that dilution reduces all the clotting factors equally, including fibrinogen, while the naturally occurring hypoprothrombinaemia presumably affects only prothrombin. Many attempts

FIG. 5.—Curve showing the relationship of prothrombin concentration to coagulation time of recalcified oxalated plasma in the presence of optimum thromboplastin. (After Quick, 1942.)

have been made to circumvent this difficulty, mainly by the use as a diluent of " prothrombin free " plasma, this being prepared by adsorbing normal plasma with Al(OH)₃ or by Seitz filtration. The results of such attempts to reproduce ranges of plasma dilution corresponding to natural prothrombin-deficient plasma have, however, been difficult to interpret. Quick (1945b) has emphasized the difference from saline-diluted plasma. Other workers (for example, Nitshe and others, 1947) have used fibrinogen solution as a diluent; and the whole complex problem—has been discussed by Conley and Morse (1948) in relation to the reliability of thromboplastin preparations.

Many modifications of this so-called one-stage method have appeared. Smith and others (1939) introduced what is known as a " bedside " technique, which is carried out with whole blood and with much the same technique as that used for the Lee and White method, except that thromboplastin is added to the blood; and Kato (1940) has devised a micro-method also using whole blood. Fullerton (1940) suggested using Russell's viper venom as thromboplastin, the satisfactory results he reported having been confirmed by Page and Russell (1941). This material has the advantage of very high activity combined with almost complete stability when the venom is dried. On the supposition that the minimum clotting time indicates the optimum thromboplastin concentration, Witts and Hobson (1942) added lecithin to the venom, thereby so greatly increasing its activity that the normal prothrombin time was about 5 seconds. The use of Russell's viper venom, however, may give dangerously misleading results in patients receiving dicoumarin.

Several workers (Quick, 1942; Shapiro and others, 1942; Link, 1943; Allen and others, 1940; Aggeler and others, 1946) have recommended the practice of making one or more dilutions of the unknown plasma in order to obtain a longer clotting time which is more critically related to prothrombin concentration. It is true that the first part of the curve is flat and that little change in clotting time is observed until the prothrombin is 50 per cent or less, but the use of dilutions obviously introduces errors and fallacies of its own, as discussed by Fisher (1947).

Other complications appear when the prothrombin concentration is so low that long clotting times are obtained by this method. It has been found by Jaques and Dunlop (1945a, b) that the calcium concen-

tration becomes very critical when low prothrombin concentrations are produced in vivo by the use of dicoumarol, or by adsorption of prothrombin by alumina in vitro, so that a slight divergence from the optimum has a considerable anticoagulant effect. Observations made by Dr. Rosemary Biggs (unpublished), extending those of Aggeler and Lucia (1938), show that thromboplastin concentration is also an increasingly critical factor as prothrombin concentration is reduced and that in such cases a slight excess of thromboplastin may actually inhibit clotting (Fig. 6).

Despite these theoretical and practical disadvantages Quick's original method provides a most valuable indication of the patient's liability to bleed, which is the main object of the majority of prothrombin estimations. The procedure has been standardized and its reliability investigated statistically by Aggeler and others (1946), but a few comments on the expression of the results might be added. In many laboratories it is the practice to express the results of a prothrombin
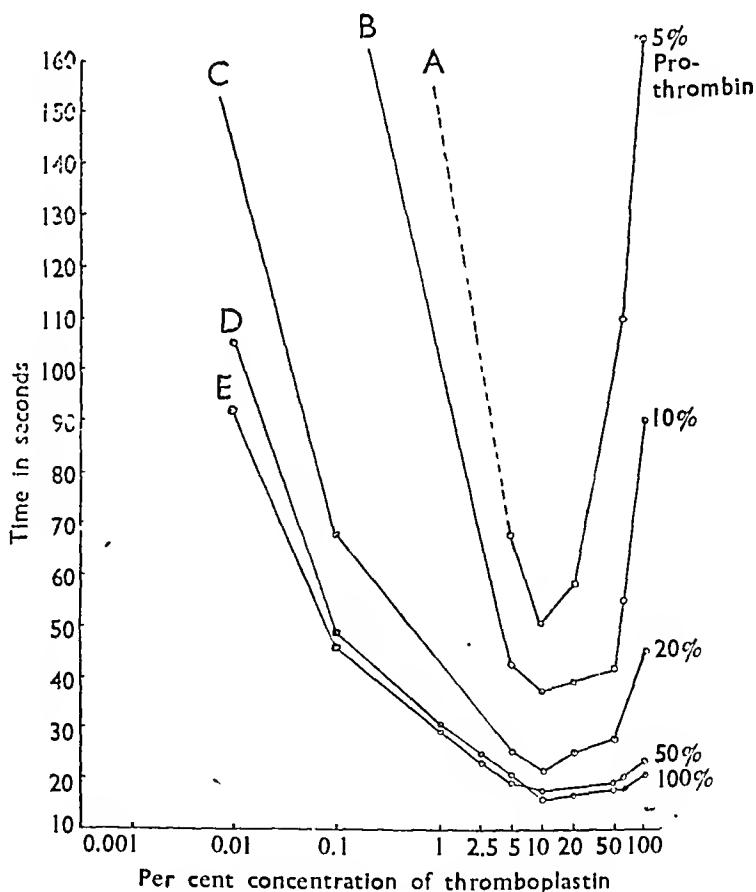
FIG. 6.—Curves showing the relationship of thromboplastin concentration to coagulation time for a number of different prothrombin concentrations ("100 per cent" thromboplastin = 1 g. acetone dried brain in 10 ml. saline). Curve A is for 5 per cent prothrombin, B for 10 per cent, C for 20 per cent, D for 50 per cent, and E for 100 per cent prothrombin. (Dr. Rosemary Biggs's data.)

determination as an "index" by means of the formula

$$\frac{\text{Normal Prothrombin Time} \times 100}{\text{Patient's Prothrombin Time}}$$

This, while convenient, gives a very misleading impression of the prothrombin concentration actually present in the patient's blood as calculated from Quick's curve. For instance, a "prothrombin index" of 33 per cent (i.e., patient's prothrombin time = 36 seconds; normal time = 12 seconds) is equivalent to 10 per cent prothrombin, and an index of 80 per cent is equivalent to 50 per cent prothrombin. Quick (1939) has suggested the use of the formula

$$\text{Prothrombin } \% = \frac{302}{\text{Prothrombin time} - 8.7}$$

assuming that the normal prothrombin time with the thromboplastin used is about 12 seconds. The use of this is preferable to the index, if the curve itself is not available. A difficulty in the use of both curve and formula is that of standardizing the thromboplastin to give a normal time of 12 seconds. The definition of the term "normal" in this respect obviously requires further investigation. In the writer's laboratory a number of curves have been constructed relating prothrombin concentration to clotting time for different levels of thromboplastin activity. By
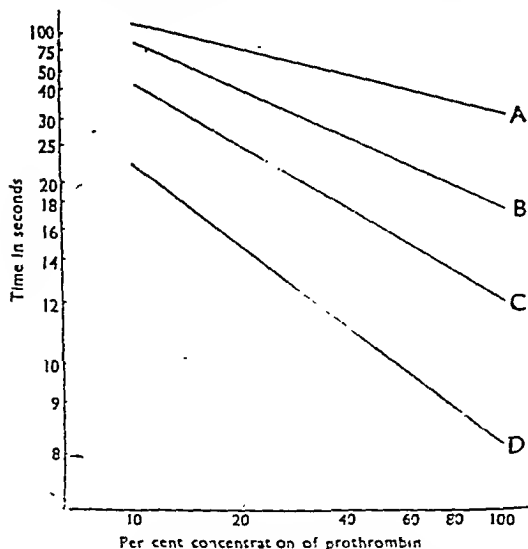
Fig. 7.—The relationship of clotting time to prothrombin concentration as it applies to Quick's method (determined by means of plasma dilutions) for 4 thromboplastin preparations of different potency, A, B, C, and D. Approximate linearity has been obtained by plotting the reciprocal of the clotting time (vertical axis) against the logarithm of the percentage prothrombin concentration (horizontal axis). (Dr. Rosemary Biggs's data.)

plotting the logarithm of the prothrombin concentration against the reciprocal of the clotting time, almost straight lines are obtained (Fig. 7). By the use of these, a given prothrombin time can be read off as concentration by interpolation, the activity of the thromboplastin used being known by testing against normal plasma. Rapoport (1947) has discussed other methods of calculating results.

It has already been pointed out that Quick's method depends upon the assumption that the *amount* of prothrombin available controls the *rate* at which thrombin is formed, this rate in turn determining the clotting time of the plasma. In fact, only about 1 per cent of the prothrombin has been converted by the time that clotting occurs in normal plasma, full conversion, even in the presence of optimum thrombokinase, taking some minutes.

It would appear more scientific to use, instead of this dynamic method, dependent as it is on unknown factors controlling the rate of thrombin formation, a static method in which all the available prothrombin is converted into thrombin, which is then assayed by means of standard fibrinogen. Such a principle is the basis of the 2-stage method originated by Warner and others (1936). In this, the unknown plasma is first clotted by a small amount of thrombin (equivalent to about 1 thrombin unit), and the fibrin so formed is removed after 15 minutes when the added thrombin has been inactivated by the antithrombin of the plasma. The resulting fluid, containing all the original prothrombin but no fibrinogen, is then diluted to 1 in 20 with oxalated saline and activated with a mixture of buffered calcium chloride and thromboplastin. Samples of this reacting mixture in which thrombin is generated are then transferred to standard fibrinogen solutions and the clotting time of this is recorded. Dilutions of the reacting mixture are made to give a clotting time of about 15 seconds, this dilution therefore containing 1 unit of thrombin per unit volume as originally defined. The number of units per ml. of original plasma is then calculated from the final dilution of the plasma, and it is assumed that one unit of thrombin is derived from one unit of prothrombin.

The process of thrombin formation under these conditions in relation to time is illustrated in Fig. 8. It will be seen that the process starts slowly, and increases in rate until the thrombin concentration reaches a maximum which, instead of remaining constant, declines. The general shape of the initial part of the curve suggests an autocatalytic reaction (see Astrup, 1944), which is of considerable theoretical interest. The important practical points are the sharply defined maximum, necessitating repeated sampling during activation for its recognition and, even more important, the destruction of thrombin by antithrombin, indicated by the falling off of the curve. The authors of the method assumed that this antithrombin action was not significant at a dilution of 1 in 20 or over, but there is little doubt that it may be, and that it constitutes a serious drawback to a method already handicapped by the practical dis-
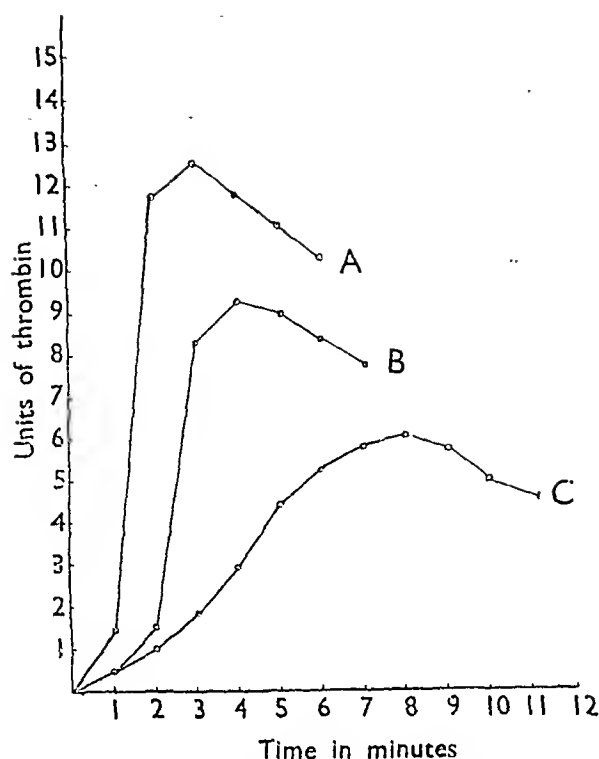
FIG. 8.—Curves illustrating the concentration of thrombin in a reacting mixture of thromboplastin, calcium, and diluted oxalated plasma, plotted against time, and for different dilutions of plasma. Curve A is for plasma diluted 1/25, curve B for plasma diluted 1/50, and curve C for plasma diluted 1/100. (Author's data.)

advantages of elaborate technique and reagents. Attempts have been made to overcome some of these disadvantages. The technical modification of Herbert (1940) provides a much simpler procedure, suitable for routine laboratory use. She makes an initial dilution of the test plasma, activates with thromboplastin and calcium, and takes sub-samples for thrombin assay, finding that the fine web of fibrin forming in the dilute reaction mixture does not interfere with the sampling. The thrombin units are read off by a calibration curve so that serial dilutions are unnecessary. Sternberger (1947) has also introduced a practical and possibly important modification by suppressing the antithrombin by the addition of alcohol. This, if successful, would eliminate the danger of loss of thrombin in slowly activating mixtures, but it has been found (personal observation) that the alcohol makes the end-point difficult to read, and it may have other unknown effects.

The 2-stage method has, of course, the academic advantage that it gives an estimate of prothrombin in absolute units and, when suitably modified, is not outside the scope of a pathological laboratory. It does not provide, however, the clinical information that the 1-stage test gives on the patient's liability to bleed.

Discrepancies Arising out of Prothrombin Estimation

The use of these two different principles for determining prothrombin on clinical and experimental material soon revealed a number of disturbing discrepancies in the results obtained. The first to appear was the relative normal prothrombin levels of different species. Quick found, for instance (1936b, 1941a), that rabbit plasma contained five times as much prothrombin as human blood, and cat blood about three times as much. Using the 2-stage technique, however, Warner and others (1939a, b) found human, cat, and rabbit plasma to have about the same prothrombin content of about 300 units per ml. It was also found by the 2-stage technique that stored blood maintained its prothrombin content for months (Warner and others, 1940) whereas by the Quick method it lost up to 90 per cent in the first week (Quick, 1943). Other discrepancies involving the prothrombin concentration in the newborn (Owen and others, 1939; Brinkhous and others, 1937; Quick and Grossman, 1939), and in jaundice, and also the level at which haemorrhage occurs (Brinkhous, 1940; Quick, 1938a) are listed in Table I.

TABLE I

DIFFERENCES BETWEEN RESULTS OF 1- AND 2-STAGE METHODS FOR ESTIMATING PROTHROMBIN

Source of prothrombin	1-stage	2-stage
Blood during storage	Rapid fall	Slow fall
Newborn babies	Sometimes normal	Consistently low
Obstructive jaundice	Low in some cases	Low in most cases
Cases beginning to bleed from prothrombin deficiency	10% prothrombin	30–40% prothrombin
Rabbit blood ..	500% of man	100% of man
Cat blood ..	300% of man	110% of man

It will be seen from this that the difference is not in the same direction in each case, the 2-stage giving lower results than the 1-stage in animals, jaundice cases, and babies, but higher on stored blood and at the haemorrhagic level. It is clear that the two methods are measuring different things, though both claim to measure prothrombin.

At first these discrepancies were considered to be due to differences in the "activity" or reaction rate of prothrombin (Warner and others, 1939b),

but later Quick (1943) made a fundamental observation relating to the discrepancy in the stored-blood estimation. He found that if a volume of plasma from which " prothrombin " had been removed by adsorption by alumina were mixed with a volume of stored plasma with a prothrombin time of 37 seconds (indicating 10 per cent prothrombin content), the prothrombin time of the mixture was 10 seconds, so that the mixture appeared to contain 100 per cent prothrombin and not the expected 5 per cent. Further work established the fact that plasma with a low " prothrombin " content following dicoumarol administration also had the effect of reducing the prothrombin time of stored plasma. From this and other evidence Quick concluded that " prothrombin " is made up of two components, a labile factor destroyed by slight degrees of heat, not absorbed by $Al(OH)_3$, and becoming inactive after a few days' storage (called at first component A), and a stable factor (component B) which retains its activity for long periods, is relatively heat-stable, is strongly adsorbed by $Al(OH)_3$, and is reduced by the *in vivo* effect of dicoumarol. In normal and dicoumarol plasma the labile factor is in excess.

Confirmation of the existence of such hitherto unrecognized factors required for thrombin production came rapidly and in some cases independently. Quick's observations were substantiated by Oneal and Lam (1945) and Munro and others (1945), but were disputed by Loomis and Seegers (1947), whose contribution will be discussed later. Feissly (1945c) and Fantl and Nance (1946a) obtained evidence of an accessory factor not included in the classical theory and which they concluded was required for the activation of prothrombin. Zondek and Finkelstein (1945) concluded that there was a thromboplastin co-factor which had been overlooked.

Meanwhile, Owren (1947a, b), working in enemy-oecupied Norway and largely cut off from events in the United States, carried out an investigation which has brilliantly illuminated the problem of additional clotting factors. This work began in 1943, when a patient with a curious haemorrhagic diathesis came to his attention. She was a woman of 29, who from infancy had suffered from a severe haemorrhagic diathesis, clinically almost indistinguishable from haemophilia except for the absence of haemarthroses. There was no significant family history, no detectable physical abnormality not attributable to the effects of haemorrhage, and no abnormal blood findings apart from a long clotting time of 25 minutes. It was found, however, that,

unlike true haemophilia, the prothrombin time by Quick's method was prolonged (70 seconds), indicating gross prothrombin deficiency. There was no reason to suspect vitamin-K deficiency, and the administration of vitamin K had no effect. Since all other clotting factors appeared to be normal and no anticoagulant could be demonstrated, the diagnosis appeared to be " idiopathic hypoprothrombinaemia." Many investigators would have been content (as were Rhoads and FitzHugh, 1941 ; Austin and Quastler, 1945) to accept this diagnosis and do no more. Owren, however, was more tenacious, and soon found that the addition of normal human plasma to the patient's plasma, even in as small a concentration as 10 per cent, would reduce the prothrombin time of the mixture to normal. Since this could not be explained by the simple addition of prothrombin, Owren tried the effect of adding normal plasma from which the prothrombin had been removed by adsorption with $Al(OH)_3$; 20 per cent of such plasma, itself incoagulable, reduced the prothrombin time of the patient's plasma to normal, thus demonstrating the presence of some factor that was not fibrinogen, since this was normal in the patient's plasma, nor prothrombin, nor calcium or thromboplastin, both of which had been ineffective in the prothrombin test. Owren called this factor which was deficient in his patient " factor 5," being the fifth clotting component. He went on to isolate factor 5 in a state of relative purity, and in an extensive series of experiments he showed that it is very labile, is not absorbed by $Al(OH)_3$, and is essential for the conversion of prothrombin to thrombin by thromboplastin and calcium. He showed that the injection of the factor into his patient reduced her coagulation time to normal. The failure by previous workers to recognize factor 5 seems to be due to the fact that, by ordinary methods of preparation, the fibrinogen and prothrombin fractions are heavily contaminated with it, so that its detection by virtue of its absence is difficult.

Quick (1947c) has not been slow to recognize Owren's factor 5 as being probably identical with his own " labile factor." Indeed, it seems probable that most of the discrepancies between the 1- and 2-stage methods are due to unsuspected variations in factor 5. The 1-stage method, being dependent as it is on the *rate* of conversion of prothrombin, is very sensitive to variations in the concentration of such an accelerator, while the 2-stage method, dependent upon the *amount* of thrombin generated, is much less so. This is one of the reasons why, for practical purposes, Quick's method gives a better indication of the patient's

liability to bleed. In the rare event of a factor-5 deficiency being suspected in a patient, factor 5 can be estimated by the method of Owren (1947a), which depends on the addition of dilutions of the test plasma to factor-5-free prothrombin, fibrinogen, thromboplastin, and calcium.

It seems to be established by weight of evidence, therefore, that what is usually regarded as prothrombin is a complex of two or more components. The objection of Loomis and Seegers (1947) was based on the fact that the prothrombin time of old plasma could be restored by the addition of fresh fibrinogen so that they considered Quick's labile factor to be a fibrinogen defect, but it is probable that their fibrinogen was contaminated with factor 5, which might thus be the active agent. The finding of Chak and Giri (1948) that the prothrombin time of stored plasma can likewise be restored by the passage of CO_2 suggests, however, the possible existence of a labile inhibitor that is developed during storage, and it is not, at the moment, clear in its implications.

Factor 5 or its equivalent has been accepted as an entity by some workers with such enthusiasm that they have discussed the priority of its discovery with considerable energy (*Lancet* correspondence, 1947). It must not be forgotten, however, that it is Nolf who has always maintained (Nolf, 1908, 1938, 1945) that five factors take part in coagulation. It is most probable that the factor called "thrombogen" by Nolf is identical with the newly described "factor 5" of Owren and "labile factor" of Quick. All three descriptions portray a relatively labile substance not adsorbed by such agents as $Al(OH)_3$, $Ca_3(PO_4)_2$, or Seitz filtration, and which is necessary for the coagulation, by thromboplastin and calcium, of a mixture of fibrinogen and the convertional "prothrombin." The credit for original recognition of this factor would thus appear to be due to Nolf.

Other factors.—Unfortunately factor 5 is not the only addition which is likely to be made to the coagulation theory. Owren (1947a) considers it necessary to postulate a derivative of factor 5 which autocatalytically activates prothrombin in the presence of thromboplastin and calcium. This is based on the observation that small subsamples of a reacting mixture of thromboplastin, prothrombin, factor 5, and calcium will clot oxalated plasma faster than it will clot an equivalent solution of fibrinogen, whereas a given amount of thrombin will clot the fibrinogen faster than the plasma. It is argued that something must be produced during the reaction that activates the prothrombin of the oxalated plasma, so that there is more available thrombin in this system than in the fibrinogen mixture. It can be shown that the thromboplastin and calcium are not active in this way in the absence of prothrombin. It is supposed, therefore, that a "factor 6" is generated that explains this autocatalytic reaction. These results are of interest, but their interpretation is not yet quite clear. Astrup (1944) obtained substantially similar results, and discussed the autocatalytic reaction at some length, being of the opinion that it is due to the activation of a plasma thrombokinase. Fischer (1935b) had made similar observations, and Bertrand and Quivy (1946)—using a photometric method for estimating clotting times—and Laki (1943) also support the necessity for postulating autocatalysis in the normal generation of thrombin, but further and carefully controlled experiments are required before the significance of this phenomenon is clear.

Quick (1947e), from studies of two families with inherited haemorrhagic diathesis associated with prolonged 1-stage prothrombin times, comes to the conclusion that in one of them the defect is due to a deficiency of prothrombin component B, already described. The other family, however, shows the deficiency of another prothrombin factor which is neither B nor the labile factor (previously called component A, very probably identical with factor 5). Quick, therefore, postulates the existence of a third prothrombin component deficient in this family, and proposed "to avoid confusion" by calling his original component A "labile factor," and the new component "component A." He suggests that it is the new component A which is reduced in vitamin-K deficiency.

Quick (1947a) has also—by means of an ingenious experiment involving the decalcification of plasma in silicone-treated tubes, by "amberlite" —shown that there is probably a co-factor required for calcium action in thrombin generation, this being independent of the other components of the prothrombin complex.

Abnormal Blood Coagulation

Artificial anticoagulants.—Of the anticoagulants used either *in vivo* or *in vitro* for therapeutic or experimental purposes, heparin has already been considered, since technically it is a naturally occurring anticoagulant. The various decalcifying agents such as citrates, oxalates, phosphates, and fluorides have been discussed in relation to the effect of calcium on the coagulation mechanism;

and the pancreatic and soya bean trypsin inhibitors have been considered from the point of view of their powerful and interesting antagonism to the action of thromboplastin.

An important group of anticoagulants are certain azo-dyes, including Chicago blue and chlorazol pink studied by Rous and others (1930), Huggett and Rowe (1933), and Huggett (1934), the latter worker considering that their action was on thrombokinase. Recent work by Astrup (1944) suggests that they are similar to heparin in their action, though they do not require the heparin co-inhibitor. Germanin, the anticoagulant action of which was noted by Mayer and Zeiss (1920), was found by Lenggenhager (1936) to act by stabilizing fibrinogen. Astrup (1944) states that this drug, and liquoid (sodium polyanethosulphonate), inhibits the thrombin-fibrinogen reaction possibly by reacting directly with fibrinogen. It has been observed by Fleming and Fish (1947) that penicillin has an anticoagulant effect, but its mode of action has not yet been investigated.

Hirudin, the anticoagulant secreted by the leech, was thought by Mellanby (1909) to be an antithrombin. This, and many other similar anticoagulant secretions produced by blood-sucking animals, have been largely neglected in modern times.

Dicoumarin.—The discovery of dicoumarin ranks, in the recent history of research on the haemorrhagic diatheses, second only in importance to the discovery of vitamin K. In 1929 and 1931 Roderick recorded that there appeared to be a prothrombin deficiency in animals suffering from toxic sweet-clover disease. Quick (1937) showed that the toxic clover hay produced a significant fall in the prothrombin level of animals to which it was fed, and, with the aid of prothrombin-time estimations as an assay method, Link and his co-workers (Campbell and others, 1941; Campbell and Link, 1941; Stahmann and others, 1941) were able to identify the toxic principle as 3,3'-methylene-bis-(4-hydroxycoumarin) and were then able to synthetize it in the laboratory.

Dicoumarin acts when given by mouth by reducing the prothrombin available for coagulation. There is a lag period after administration of from 24 to 48 hours before the reduction in prothrombin (Allen and others, 1942), suggesting an interference with production rather than a direct effect on existing prothrombin. Vitamin K given with dicoumarin prevents the fall in prothrombin (Shapiro and others, 1943a : Davidson and others, 1945), but once the hypothrombinaemia is established vitamin K has little effect (Allen and others, 1942 ; Davidson and Macdonald, 1943 ; Meyer and others, 1942).

Quick (1947c) considers that dicoumarin causes a reduction of component B of prothrombin, but its mode of action is unknown. Its relation to the salicylates has been put forward by Lester (1944), and there are points of similarity between its structure and that of vitamin K which might suggest a possible competition in which the inactive dicoumarin displaces vitamin K (Fig. 2). A number of workers (Link and others, 1943 ; Meyer and Howard, 1943 ; Shapiro and others, 1943b ; Rapoport and others, 1943) have reported an effect similar to that of dicoumarin resulting from the prolonged administration of salicylates in man and animals.

Naturally occurring coagulation defects.—There is a group of haemorrhagic diatheses in which the primary cause of the abnormality is inefficient blood coagulation. This may be a result of an inborn, often hereditary, defect, or of an acquired deficiency of some necessary factor, or it may be secondary to a disease process. In general, the clinical manifestations of the different clotting defects are similar. The patient is liable to bleed for long periods of time from, or into, any tissue of the body in which the vessels are damaged by trauma or disease. In consequence, not only is there a danger of death from haemorrhage, but the effects of large effusions of blood into superficial or deep tissues, into joints, the nervous system, or internal organs, may be crippling or even fatal. There is thus a *generalized* haemorrhagic diathesis, in distinction to that of the purpuras, which is usually localized to the skin and mucous membranes (Macfarlane, 1941). As regards pathological investigations, the platelet count, the bleeding time, and the capillary resistance tests are typically normal, the coagulation defect being the only positive finding. Table II shows a classification of these conditions based on the coagulation defects thought to be responsible.

Haemophilia.—Many attempts have been made in the last 50 years to settle the problem of the slow coagulation of haemophilic blood, and to many of the investigators concerned it seemed that a solution had been found only to be refuted by others. The earliest conclusions, curiously, were nearer the most recent views than those that intervened. Sahli (1905) thought that thromboplastin was lacking in this condition, a view supported by the observation of Morawitz and Lossen (1908) that haemophilic blood clotted rapidly on

TABLE II

A CLASSIFICATION OF THE HAEMORRHAGIC DIATHESES DUE TO DEFECTIVE COAGULATION

Condition	Causation			Coagulation defect
Haemophilia	Hereditary			Thromboplastinogen deficiency
Pseudo-haemophilia	Idiopathic or secondary			
Hypoprothrombinaemia	Idiopathic or hereditary			Component A deficiency
	Vitamin-K deficiency:			
	1. Mal-absorption	Jaundice : sprue		
	2. Congenital	Neonatal		
	3. Mal-utilization	Liver disease		
	Hereditary: idiopathic			Component B deficiency
	Dicoumarin poisoning			
	Idiopathic			Factor 5 deficiency
Afibrinogenaemia	Hereditary			Fibrinogen deficiency
	Liver disease			

the addition of tissue extract, suggesting that the rest of the clotting mechanism was intact.

These observations were extended by Fonio (1914), who showed that normal platelets clotted haemophilic blood in a normal time. Attention was thus directed towards the platelet function in haemophilia, and it was suggested by Minot and Lee (1916) that they were functionally defective in this condition, a conclusion supported by Birch (1932).

It was soon pointed out, however, that these experiments, involving the transfer of normal platelets to haemophilic plasma and *vice versa*, might have other interpretations, and that a plasma factor contaminating the platelets might be the active agent (Eagle, 1935b; Patek and Stetson, 1936). The latter workers, and later Patek and Taylor (1936), showed that small amounts of platelet-free normal plasma were effective in clotting haemophilic blood, even in high dilutions and after filtration, thus explaining the familiar beneficial effect of transfusion of normal blood in haemophilia. Fractionation of the plasma showed that the active factor was associated with a globulin, a conclusion reached independently by Bendien and Van Creveld (1937). Considerable progress has been made along this line by a large group of workers in the United States, and it has been found that the factor in normal plasma effective to clot haemophilic blood is present in Cohn's Fraction 1 (Cohn, 1946), which contains also fibrinogen and some prothrombin. The factor is relatively heat-stable, so that fibrinogen and prothrombin can be destroyed without loss of activity (Lewis and others, 1946a). The administration of small quantities of this material to haemophilic subjects materially, if temporarily, reduces their clotting time (Minot and others, 1945; Lewis and others, 1946b) without inducing a refractory state sometimes seen as a result of repeated blood transfusions (Munro and Jones, 1943). It may therefore be assumed that in haemophilia there is a lack of a soluble plasma factor, associated with the globulin fraction, and probably related to, or identical with, the plasma thromboplastins already discussed. Reduced activity of this factor could explain the slow clotting of haemophilic blood which is due to the very small amount of prothrombin converted during the clotting process (Brinkhous, 1939), and the normal "prothrombin time" observed by Quick, Stanley-Brown, and Bancroft (1935) when thromboplastin is added.

The platelets, however, have once more assumed some indirect importance in the understanding of this problem. In 1941(b) Quick introduced a diagnostic test for haemophilia which depended on the

fact that centrifuging oxalated haemophilic blood increases the clotting time of the recalcified plasma to a much greater proportionate degree than does the similar treatment of normal blood. Though the exact implication of this reaction is not clear, it suggests that haemophilic plasma is abnormally dependent on the presence of platelets. This particular type of investigation has now been extended by Brinkhous (1947). Using platelet-free plasma samples obtained without any contact action by centrifuging in silicone-lined tubes, he made the following observations:

1. Neither normal nor haemophilic platelet-free plasma will clot on contact with glass, either separately or mixed together.
2. The addition of normal or haemophilic platelets to the haemophilic plasma did not result in clotting in glass.
3. The addition of *both* platelets (normal or haemophilic) and a small amount of normal plasma to the haemophilic plasma resulted in rapid coagulation in glass.

Brinkhous therefore concludes that both platelets and a plasma factor are essential for thromboplastic activity, and that the plasma factor, which he thinks is a " thrombocytolysin " that normally disrupts the platelets to furnish thromboplastin, is lacking in haemophilia. Quick (1947b) has made essentially similar observations by a similar technique, but concludes that the plasma factor is a thromboplastin-precursor (thromboplastinogen) normally activated by a platelet factor, the former being deficient in haemophilia. The writer has already suggested that the platelet factor may not be an activator but a lipoid co-factor.

Whether this defect is due to deficiency of " thromboplastinogen " or to the presence of an inhibitor is not yet clear. Tocantins (1943a, b; 1946) favours the presence of an " anticephalin " in excess. Feissly (1944) also finds an anticoagulant excess in haemophilic blood, associated with the albumin. On the other band it is curious that there are persistent reports of some defect in the fibrinolytic system of haemophilic blood (Tagnon and others, 1942; Feissly, 1942; Macfarlane and Biggs, 1948), and the possibility that plasmin may be an activator of the thrombokinase complex cannot be ruled out. It may be mentioned that factor 5 is normal in haemophilia (Owren, 1947a).

Pseudohaemophilia.—The term pseudohaemophilia should be reserved to describe the states in which there is a clotting defect indistinguishable from that found in haemophilia, but which, for genetic reasons, cannot be regarded as the true condition. Joules and Macfarlane (1938) described such a diathesis acquired by a woman in late middle life. In no way could the clotting defect be shown to vary from that of true haemophilia, and apart from the history the clinical resemblance was complete. A similar case has been described by Maddison and Quick (1945). It has also been observed by the writer (unpublished) to occur as a complication of conditions with abnormal globulins, such as multiple myelomatosis, and as Hodgkin's disease. The use of the term pseudohaemophilia to describe cases of athrombocytopenic purpura, a condition which has no clinical, genetic, or haematological resemblance to haemophilia but which is still so described by recent writers (Estrer and others, 1946; Perkins, 1946), is to be deplored.

Hypoprothrombinaemia.—The historical background to the recognition of hypoprothrombinaemia and the existence of vitamin K has already been briefly outlined, and an admirable and detailed account of the conditions in which it occurs will be found in Quick's monograph (Quick, 1942).

The most important cause of hypoprothrombinaemia is vitamin-K deficiency, which may arise in several ways. In obstructive jaundice and biliary fistula the absence of bile salts reduces the absorption of the natural fat-soluble vitamin K from the intestine, with consequent lowering of the prothrombin content of the patient's blood and the development of the characteristic bleeding tendency that so often complicated this type of jaundice in the past. In haemorrhagic disease of the newborn there is, apparently, a congenital deficiency of vitamin K which can be prevented by the prophylactic use of the vitamin (see Lehmann, 1944). A less familiar cause of vitamin-K deficiency is the intestinal absorption abnormality associated with sprue, steatorrhoea, or chronic diarrhoea (Kark and others, 1940; Collins and Hoffmann, 1943). The haemorrhagic diathesis in such cases may occasionally develop rapidly and lead to diagnostic confusion. In liver disease with impaired function the prothrombin-deficiency is apparently due to failure to utilize vitamin K, and, unlike the other conditions mentioned, there is little response to its administration.

In animals, prothrombin deficiency may occur as a result of poisoning by feeding stuffs containing dicoumarin, a condition that has already been mentioned in relation to the discovery of the toxic factor. The now prevalent use of this material in human beings for therapeutic or prophylactic purposes is an important source of hypoprothrombinaemia, though, strictly, it cannot be called a " naturally occurring " condition. The effect of dicoumarin, being delayed and to some extent cumulative, is not always easy to control. In consequence there are many instances of the development of alarming haemorrhagic manifestations,

such as bleeding from operation sites, from mucous membranes, into the tissues, or from the kidneys (Allen and others, 1942 ; Crawford and Nassim, 1944 ; Shlevin and Lederer, 1944). Allen and others (1942) found that vitamin K did not reverse the effect of dicoumarin in such cases, though blood transfusion was more effective.

In controlling dicoumarin therapy, and in the detection of hypoprothrombinaemia in general, it is the writer's experience that Quick's prothrombin method performed with a brain thromboplastin gives clinically the most useful results. Prothrombin concentration is expressed as a percentage of normal by the use of a number of the curves already described. It is important to remember that with low concentrations of prothrombin such as are met with in dicoumarin poisoning, the concentration of calcium and thromboplastin becomes very critical, and that occasionally an excess of either may so delay the clotting that Quick's test has a longer time than the simple coagulation time of the same blood sample (Macfarlane, 1942 ; Fantl and Nance, 1947). Russell's viper venom has been found unreliable as a thromboplastin in assaying the effect of dicoumarin, since the prothrombin time may be considerably shorter than with brain extract and a dangerous overdosage of the drug may be given, an effect also observed by Lempert (1948). It must also be remembered that patients with a moderate hypoprothrombinaemia which is above the level at which bleeding occurs may be precipitated into a severe haemorrhagic diathesis by blood loss. The prothrombin concentration of the blood, already deficient, may be lowered disastrously by the dilution of the blood volume that follows severe bleeding, such as that occurring at operation or delivery.

The cases of so-called idiopathic hypoprothrombinaemia as described by Rhoads and FitzHugh (1941) and Austin and Quastler (1945) are probably examples of a deficiency of one of the newly recognized " prothrombin " components such as factor 5. Owren's (1947b) use of the term " parahaemophilia " for this condition seems to cause confusion, in particular since he has identified the precise deficiency in his own case. Quick's (1947a) two families with component-A and component-B deficiency respectively seem to be examples of inherited defects corresponding to those produced by vitamin-K deficiency, which apparently reduces component A, and dicoumarin poisoning which reduces component B.

Fibrinopenia.—Fibrinopenia may occur as a congenital absence of fibrinogen, possibly inherited as a recessive character (Macfarlane, 1937a) or secondarily to severe liver disease. Both are rare conditions, only 3 cases of the congenital form having been described in this country (Macfarlane, 1937a ; Witts, 1942 ; Henderson and others, 1945). The subject has been recently reviewed by Yeager and others (1947).

When the absence of fibrinogen is complete, as was the case with the English patients, the blood is, of course, incoagulable by any means except the addition of fibrinogen. The rest of the clotting system appears to be intact, however (Macfarlane, 1937a), though thrombocytopenia may be associated with the disorder. It is curious that these patients are less severely incapacitated by their abnormality than is usually the case with haemophilia, despite the fact that any participation of blood coagulation in whatever mechanism prevents fatal bleeding is impossible.

The fibrinopenia secondary to some disease process is usually not complete so that diagnosis may be more difficult, since the clotting time is normal. The poor quality of the clots should be obvious, however, and a fibrinogen determination will reveal the defect.

Increased fibrinolysis.—It has been suggested (Reimann, 1941) that inherent fibrinolysis of the clots formed in certain cases may be the cause of a haemorrhagic diathesis. The evidence put forward is not convincing, and even in cases with active fibrinolysis no haemorrhagic diathesis has been observed by the writer that could be attributed directly to its action. Bacterial contamination, however, may activate plasmin to an extreme degree and produce considerable proteolysis that may be one cause of so-called secondary haemorrhage.

Circulating anticoagulants.—It has already been stated that anaphylactic shock will cause an outpouring of heparin in animals and possibly in man. A similar effect has been observed by Allen and Jacobson (1947) in animals exposed to irradiation, an observation of importance in view of the increasing risk of such exposure in modern life.

Naturally occurring anticoagulants in demonstrable form as a cause of bleeding are of extremely rare occurrence, though cases have been described by Lozner and others (1940) and Fantl and Nance (1946b).

Hypercoagulability of the Blood

1. Artificial coagulants.—It is understandable that in the past, when the specific deficiencies now recognized to be responsible for coagulation defects were not understood, recourse should have been had to many strange substances thought to promote coagulation. Comparatively few agents have in fact the effect of hastening true coagulation other than those already partaking in the natural process. In recognition of this, attempts were made to extract these natural coagulants, and many proprietary preparations were derived from platelets, leucocytes, brain, muscle, lung, glands, milk, and so on. Since these were often intended to be given parenterally it is perhaps fortunate that few of them possessed the powerful coagulant effect claimed for them, so that if no benefit was derived from their use little harm came either. It soon came to be recognized that in haemophilia,

to the treatment of which these efforts were mainly directed, only the transfusion of fresh normal blood, or the globulin fraction derived from it, is effective in lowering the clotting time.

The use of calcium for diminishing the risk of haemorrhage is still prevalent, however. It is apparently based on the misleading statement by Wright (1893) that calcium reduced the clotting time of haemophilic blood, and there seems to be little other evidence to support its use.

For local application, two natural products, thrombin and fibrin, have proved themselves to be useful. There are now several preparations of thrombin which are extremely active and will produce coagulation within a few seconds. One of these is derived from the interesting "rabbit-clotting globulin" described by Parfentjev (1941) and Taylor and others (1941), and subsequently produced commercially. It must be stressed, however, that no matter how powerful the coagulant its mere surface application to a bleeding point is useless for haemostatic purposes. The flow of blood merely washes the coagulant away to form ineffective clots at some point removed from the site of haemorrhage. It is essential in addition to control the bleeding by temporary pressure to give the mixture of blood and coagulant time to clot *in situ.*

The haemostatic efficiency of a blood clot is increased if it is reinforced by a dressing or light plug. The disadvantage of such foreign material is that it has eventually to be removed, a process often resulting in a recurrence of haemorrhage. The use of preformed fibrin in the form of sheets or " foam " is a considerable advantage in this respect, as it serves as a dressing that is naturally disposed of during healing (Macfarlane, 1943). Such fibrin preparations have now been greatly improved by American workers and have been used with success in a number of conditions (Bering, 1944 ; Ferry and Morrison, 1944 ; Woodhall, 1944). Other digestible haemostatic dressings of artificial nature, such as gelatine sponge (Light and Prentice, 1945) and soluble cellulose (Seegers and Doub, 1944), have also been reported to be efficient.

Of the artificial coagulants, some, such as citrate, egg albumin, peptone, pectin, gum arabic, and others, have been given parenterally. Citrate, paradoxically, seems to have some slight and transitory coagulant effect (Neuhof and Hirschfeld, 1922), but very large transfusions of citrated blood to exsanguinated patients have, in the writer's experience, been associated with depressed coagulation. An egg albumin preparation was

developed by Timperley and others (1936) for the treatment of haemophilia, but their early results have not been substantiated.

Of artificial coagulants for local use, the venom of Russell's viper, physiologically resembling a thromboplastin, has been found effective (Macfarlane and Barnett, 1934 ; Macfarlane, 1935) with the same reservations that apply to the use of thrombin. Other snake venoms have a coagulant action (Barnett and Macfarlane, 1934), and Rosenfeld and Lenke (1935) have used that of the Australian tiger snake as a local haemostatic.

Many other substances, mainly used out of respect for tradition, have been employed as local haemostatics, and it would be laborious and out of place to consider them here. An account of many of them is given by Pickering (1928). Some, such as ferric chloride and turpentine, are not true coagulants but precipitants of plasma protein, and they have a dangerously necrotizing effect on the. tissues, so that their use is absolutely contraindicated in haemophilic patients in whom healing must be encouraged by all possible means. The cautery has a similar effect and should be avoided in haemophilic patients for the same reason.

2. *Intravascular coagulation.*—The clotting of blood within damaged vessels and those immediately adjacent to a traumatized area is, of course, a physiological response to injury and a safeguard against blood loss. Coagulation in such vessels occurs because the local production of coagulant factors overwhelms the local concentration of anticoagulant factors. This process extends until the junction of the affected vessel with one carrying flowing blood is reached, when the clotting factors are so diluted as to be ineffective. Similarly, on isolated areas of damaged endothelium there will be deposition of platelets followed by fibrin to build up the familiar white thrombus, but so long as the blood flow is maintained massive intravascular coagulation will not occur.

The uncontrolled extension of this physiological process or its appearance in remote parts of the body is a familiar hazard of post-traumatic and post-operational states, and of certain disease processes. Such extensive thrombosis may restrict the blood supply of a limb, or of a vital tissue, as in mesenteric, coronary, or cerebral thrombosis, and detachment of a clot may cause pulmonary or other embolism.

Since it is true to say that many more patients die of thrombosis and its effects than of inefficient coagulation, it is clear that the problem is of greater social importance than the haemorrhagic diatheses and would seem to deserve greater attention in this review. However, this mysterious and

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unpredictable occurrence apparently is not primarily due to an abnormality of the blood-clotting mechanism, but to the combination of tissue damage and circulatory stasis that may displace the clotting–anticlotting equilibrium in favour of coagulation.

A further cause of thrombosis is infection by certain organisms. It is well known that most pathogenic strains of *Staph. aureus*, for instance, produce a toxin capable of rapidly clotting human blood. Since it is equally effective if the blood is oxalated, it was assumed to act as a thrombin. Smith and Hale (1944) have investigated the action of this toxin and have shown that it requires the presence of a co-factor, at the moment unidentified, that exists in most, but not all, samples of human plasma.

Many of the snake venoms already mentioned are powerful blood coagulants, and the rapidly lethal effect in small animals and the local gangrene in man following bites by such snakes is probably related to intravascular coagulation (Macfarlane, 1937b).

So far, no abnormality of the blood has been established as a reliable indication of impending pathological thrombosis. It is known that trauma, as such, increases the clotting efficiency of the blood. It is well known, for instance, that the platelet count increases (Tocantins, 1938) and that there is a rise in fibrinogen and the sedimentation rate, and a fall in the coagulation time. These changes, however, are physiological rather than pathological, and no quantitative estimate of their magnitude seems to have been related to the incidence of thrombosis.

Platelets and thrombosis.—There are, however, certain lines along which progress is being made. The importance of the behaviour of the platelets in thrombus formation has been stressed since the time of Bizzozero (1882). Recently the " stickiness " of platelets, obviously an attribute that controls their tendency to adhere to foreign surfaces, has been studied quantitatively by Wright (1941, 1942, 1944). She has found that in conditions associated with an increased platelet count, such as parturition and surgical operation, there is an increase in the adhesiveness. This may be related to the fact that young platelets are more adhesive than old ones and that rapid production from the marrow increases the proportion of young platelets. She has also found that anticoagulants, such as heparin and chlorazol-fast pink, decrease the adhesion of platelets as measured by her method. A similar decrease of platelet adhesion following dicoumarin adminis-

tration has been observed by Spooner and Meyer (1944). The nature of platelet adhesion is at present unknown. It was supposed by some authors (Lenggenhager, 1936 ; Wright, 1946) to be the result, rather than the cause, of coagulation, being due to a film of fibrin forming on the platelet surface. The demonstration of platelet adhesion by Pinniger and Prunty (1946) in the blood of a case of congenital total absence of fibrinogen appears to dispose of this explanation.

Other blood changes in thrombosis.—Hirschboeck and Coffey (1943) have utilized a clot retraction-time estimation which they claim gives results related to the danger of thrombophlebitis and pulmonary embolism, and which, subject to confirmation, are of interest.

Waugh and Ruddick (1944a, b) have made an interesting study of the anti-heparin effect they have observed in the blood of patients kept at rest in bed and in acute infection. They find that heparin has less anticoagulant effect on the blood of such patients *in vitro* than in normal blood, an observation that is worth extending.

The work of Lyons (1945a, b) on the existence of a more reactive fibrinogen (fibrinogen B) that may be increased in conditions associated with thrombosis appears to be of considerable promise and should be followed up without delay.

Finally, it may be said that there are many factors possibly related to the problem of thrombosis that have received very little attention. Fibrinolysis may be a process that normally limits the extension of intravascular clots, and it would be instructive to follow the development of the fibrinolytic reaction in relation to thrombosis. A systematic assay of the normal anticoagulant factors might also yield information of considerable value.

The prevention of thrombosis.—Once thrombosis is established very little can be done apart from attempts to prevent its extension. In this respect, and more particularly as a prophylactic measure in post-operative and post-partum cases, the use of the anticoagulants heparin and dicoumarin has been extensive and apparently successful. The pioneer work of Murray and others (1937), Best and others (1938), and Solandt and Best (1938) established that heparin was an agent preventing platelet agglutination and thrombus formation on damaged intimal surfaces, both active and non-toxic *in vivo*. Thereafter, heparin has been used on a large scale (Murray, 1940 ; Crafoord, 1939 ; Crafoord and Jorpes, 1941) as a therapeutic and prophylactic measure, and in vascular surgery. Heparin has a rapid effect which is of short duration, and in consequence it is usually advisable to give it by continuous intravenous drip (Quick, 1942) or by four, five, or more intravenous

injections daily (Crafoord, 1939), if the clotting time is to be maintained at a reasonably high level. This practical disadvantage might be overcome by combining heparin with some slowly absorbed base. Attempts in this direction have been made by Loewe and Rosenblatt (1944) and Evans and Boller (1946), but the local injection of such preparations has its own disadvantages. As regards dosage, this should be controlled by Lee and White clotting-time estimations, a time of about 20 or 30 minutes being desirable. " Units " of heparin are unfortunately still variable quantities, though it has been recommended by the Toronto workers (Best, 1940) that it should be fixed at 1/100 mg. of their pure barium salt of heparin. An initial dose of about 10,000 units is usual for an adult, subsequent doses being regulated by the patient's response.

Since ordinary preparations of heparin have a transitory effect, untoward haemorrhage can usually be controlled by merely stopping adminis- tration. Cerebral haemorrhage, however, is a real danger in patients with infective endocarditis treated with heparin (Witts, 1940).

Dicoumarin.—The difficulty of maintaining the effect of heparin, which is a relatively safe drug, has led to the use of the slowly acting, but more dangerous, dicoumarin. Introduced by Butt and others (1941) and Bingham and others (1941), it has had an extensive clinical application with appar- ently satisfactory results from the therapeutic point of view (Allen, 1947 ; Zilliacus, 1946). It must be mentioned that the incidence of thrombosis in the treated cases cannot be compared legitimately with the incidence of thrombosis in cases treated in the days before anticoagulant therapy was instituted, since other factors, such as avoidance of immobil- ization, reduction of infection, and other improve- ments may also have reduced the liability to thrombosis. Such comparisons, however, are made by the authors quoted. Not all authors agree, moreover, as to the therapeutic value of dicoumarin (Wasserman and Stats, 1943) or as to the theoretical basis for its use (Moses, 1945).
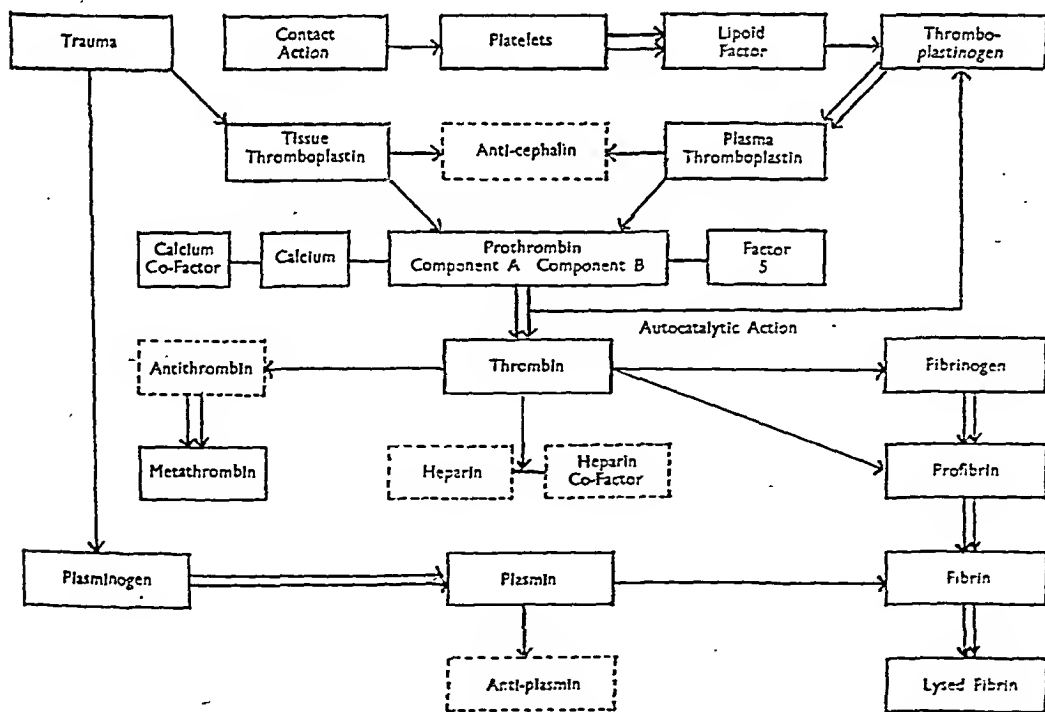
FIG. 9.—A diagrammatic synthesis of the factors probably concerned in coagulation, and their interrelationship. A single arrow signifies "reacts with." A double arrow signifies "produces." A line joining two factors signifies "in conjunction with," more precise information in this case being not available. Anticoagulant factors are outlined with a dotted line.

As regards dosage, it is usual to begin with 5 mg./kilo body weight on the first day, followed by 1.5 mg./kilo daily (Quick, 1942) until the prothrombin concentration as measured by Quick's method is between 20 per cent and 30 per cent. Below 10 per cent there is a serious risk of bleeding, which once established may be difficult to stop even by large doses of vitamin K and blood transfusion. Control of the action of the drug is not always easy. There is a variable lag period, so that there may be a long delay before a significant fall in prothrombin, and continuing depression after cessation of administration, both complicated by variations in the susceptibility of individual patients. The technical difficulties of prothrombin determination in patients receiving the drug have already been noted.

Conclusion

From the rather confused collection of facts, observations, and opinions discussed in the foregoing review it is possible by a (perhaps unjustified) selection to build up a composite picture of the coagulation mechanism. This, unfortunately, is considerably more complicated than the older conceptions had visualized. It is illustrated diagrammatically in Fig. 9, which is a synthesis embodying those factors which seem reasonably well established. It will be seen that coagulation is initiated by admixture with tissue fluid, which provides active thromboplastin, or, what is probably more important, by contact with a foreign surface. This causes rupture of platelets with the consequent availability of some activating factor, or co-factor, which reacts with the thromboplastinogen (using Quick's term) to form active thromboplastin, which in turn reacts with the "prothrombin complex." This complex is, for the moment, shown as a combination of calcium, calcium co-factor, prothrombin components A and B, and factor 5. The exact relationship of these components to each other and to thrombin production is not clear at present, and a more specific designation is unjustified. The presence of all of them, however, is required for adequate production of thrombin under the influence of thromboplastin. Once formed, thrombin reacts with fibrinogen, possibly by the sulphydryl mechanism already discussed. It is possible that some intermediate fibrinogen product is formed, though whether this is "profibrin" or "fibrinogen B" or whether these two are identical is not yet established; the supposition that there are two different thrombins does not seem sufficiently supported to justify their inclusion. The so-called "autocatalytic reaction," illustrated in the diagram as the activation of

thromboplastinogen by some effect of thrombin formation, is largely speculative and is merely one explanation of an observed phenomenon. Owren considers that an actual factor, "factor 6," is produced which activates prothrombin directly. The arguments for and against this contention are too involved to pursue here, but it can be said that since the activity ascribed to factor 6 might well be due to one of the other known factors involved, or even to a physical effect, and since the assumption of its existence complicates, rather than simplifies, the concept of thromboplastic action, the principle of Ockam's razor, never very popular with the constructors of coagulation theories, should reluctantly be applied.

The diagram illustrates also the probable inhibitors of coagulation, and it should be realized that only when the rate of production of the clotting factors exceeds the rate at which they are neutralized by inhibitors can coagulation occur. The fibrinolytic system is included, partly for the sake of completeness, since the disposal of blood clots is a physiological necessity, and partly as a reminder that it may play a much more important part in the coagulation mechanism than is now apparent.

It may seem curious that blood coagulation, which is after all a subsidiary and non-essential fragment of total bodily activity, should involve the multiplicity of factors illustrated, and a complexity which grows rather than resolves with increasing knowledge. Perhaps it is that in this particular subject the rather intensive investigation it has received has merely revealed more clearly the almost limitless complexity that underlies even the simplest of biological processes. It may be, however, that the conception of separate interacting factors is wrong in principle, being based on the artificial reactions of portions of plasma removed by force. Future workers may possibly agree with Pickering, who wrote twenty years ago that "instead of postulating a large number of bodies reacting in unknown ways, the plasma is seen as a unit capable of easily reacting to foreign substances by its unsatisfied side-chains, but returning to a normal condition when the foreign material has been eliminated."

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THE DETERMINATION OF PACKED CELL VOLUME FROM BLOOD AND PLASMA GRAVITIES IN INDIAN SOLDIERS

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Phillips and others (1945) recommended the use of standard copper sulphate solutions for determining the specific gravity of whole blood and of plasma. From these two values they calculated the blood cell volume using Ashworth and Tigertt's (1940–1) equation:

$$(1) \qquad CV = 100 \times \frac{Gb - Gp}{Gc - Gp}$$

where CV is the volume in ml. of packed cells in 100 ml. of blood, and Gb, Gp, and Gc are the specific gravities of the whole blood, plasma, and packed cells respectively. For Gc the normal value of 1.0970 was assumed.

The object of this investigation was to find out how far this method could be employed usefully in India Command in place of the usual haematocrit technique, which requires equipment not always available. Assessments of mean corpuscular volume based on an inspection of dry blood films or of the cells in the counting chamber cannot be expressed in figures, and it has thus been difficult to obtain information as to the improvement or deterioration in individual cases or to compare the blood state of different groups of population.

Seventy-five samples of venous blood were taken, the red cell concentration, the haemoglobin content, and the packed cell volume were measured, and the mean corpuscular volume and mean corpuscular haemoglobin concentration calculated. The specific gravities of the whole blood and plasma were estimated by the copper sulphate method and the packed cell volume was calculated according to equation (1). The subjects were Indian soldiers, most of whom were healthy. The cases of severe anaemia were hospital patients suffering from post-dysenteric debility.

Methods

Venous blood was withdrawn without stasis into syringes sterilized not less than twenty-four hours previously by hot liquid paraffin; 2.5 ml. was measured into a neutral glass tube marked at this volume and containing 2 mg. per ml. of a 3:2 mixture of ammonium and potassium oxalates. All estimations were done within three hours after the collection of the blood.

Haemoglobin.—A Sahli-Adams " Hemometer " was used with square, non-fading, plano-parallel double-glass standards. This apparatus was restandardized against bloods the haemoglobin content of which was determined by the method of King and others (1944), in which an alkaline haematin standard prepared from crystalline haemin is employed.

Red blood cells.—A Spencer's bright-line counting chamber with the improved Neubauer ruling was used. Each count was performed twice in two dilutions prepared in two different mercury calibrated pipettes.

Packed cell volume.—Wintrobe's " Hematocrit " tubes of 3 mm. bore were used. The samples were centrifuged for one hour in a Clay-Adams " Senior Model " centrifuge at 2,500 r.p.m.

TABLE I

DISTRIBUTION OF THE HAEMOGLOBIN AND MEAN CORPUSCULAR VOLUME LEVELS AMONGST THE 75 INDIAN SOLDIERS INVESTIGATED

Number of cases		Haemoglobin in g.%						
		Below 5	5–7.9	8–9.9	10–11.9	12–13.9	14–15.9	16 and above
		6	6	9	13	14	22	5
Mean corpuscular volume in cu.μ	Total number							
Below 80	4	1		2			1	
80–99	33	2	1	1	3	4	18	4
100–109	13	1	1		2	6	2	1
110–129	15	2		3	6	3	1	
130–160	10		4	3	2	1		

Specific gravity.—Drops of whole blood and plasma were delivered from a capillary pipette into standard copper sulphate solutions varying in specific gravity by 0.001. Values falling between those of two adjacent standard solutions were interpolated to the nearest 0.0002 specific gravity.* While the effect of temperature is the same on the gravity of copper sulphate solutions and of plasma, this has not yet been proved to apply to whole blood also, and as the laboratory temperature varied between 12 and 35° C. it was noted for each measurement.

Calculation of CV.—From the specific gravities of whole blood and plasma, CV was calculated using the above quoted equation. The added oxalates do not distribute themselves absolutely equally between cells and plasma, but this difference has only a small effect on the calculated results. The specific gravity values are given in Table II, and if wanted the corrections for oxalates according to Phillips and others (1945) can be introduced at any time. Gc was taken to be 1.0970.

Results

Table I shows the distribution of the haemoglobin and mean corpuscular volume levels amongst the seventy-five soldiers investigated.

If one classifies a blood haemoglobin content of above 14 g. per cent as normal, one between 12 and 13.9 g. per cent as showing a tendency towards anaemia, and one below 12 g. per cent as definitely anaemic, then 36 per cent of the subjects can be

regarded as normal, 19 per cent as mildly anaemic, and 45 per cent as showing pronounced anaemia. Half the cases had a mean corpuscular volume of below 100 cu. μ as calculated from haematocrit values : of the other half, 33 per cent were mildly and 66 per cent severely macrocytic, having mean corpuscular volumes between 100 and 109 and between 110 and 160 cu. μ respectively.

Table II shows the CV values in all seventy-five cases and compares the results obtained by centrifuging and by calculating from Gb and Gp.

The haemoglobin content for each sample is given, and the table is subdivided into sections according to the mean corpuscular volume. It will be seen that the CV value calculated from the specific gravity is usually lower than the one obtained by measuring the packed cell volume by centrifuging. The Gc of 1.0970 used for the calculation was obviously too high for our samples. It has to be pointed out that the originators found Gc to be 1.0970 in normal heparinized bloods : it should therefore be expected to be higher rather than lower in our oxalated suspensions. Had a correction for oxalates been introduced the differences between the two CVs would be slightly greater still.

There is an association between the differences in the two CVs and the mean corpuscular volume. This difference is significant since $0.05 > P > 0.01$ when tested by analysis of variance. The calculated CV is on the average 5.50 less in the bloods with a mean corpuscular volume below 100 cu. μ : the difference is -4.52 when the mean corpuscular volume is between 100 and 109 cu. μ and only -3.16 in the samples with mean corpuscular volumes of 110 cu. μ and above.

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* The specific gravity of blood or plasma is measured by dropping the fluid into copper sulphate solutions varying by 0.001 in gravity. For example, if a plasma drop falls in a solution of s.g. 1.024 and rises in one of s.g. 1.025, the gravity of the plasma is between 1.024 and 1.025. If it rises in the one with the same speed as it falls in the other the plasma gravity is taken to be 1.0245. If it rises more quickly in the 1.025 solution than it falls in the 1.024 solution, the gravity is between 1.024 and 1.0245. If it is stationary in the 1.025 bottle the gravity is 1.025, if it is almost stationary but falls very slowly it is taken to be 1.0242 and so on.

TABLE II

COMPARISON BETWEEN CV FOUND BY CENTRIFUGING AND CALCULATED FROM
SPECIFIC GRAVITY VALUES OF BLOOD AND PLASMA

Haemoglobin in g.%	Temp. in ° C.	Gb	Gp	CV found by haematocrit	CV calculated from sp. gr.	Difference in % $\frac{CV \ (sp. \ gr.)}{CV \ (haematocrit)}$
			Mean corpuscular volume below 80 cu. μ			
4.5	19.2	1.0380	1.0282	20.0	14.2	−29
8.0	24.9	1.0480	1.0306	32.9	26.3	−20
8.5	13.9	1.0432	1.0265	29.0	23.7	−18
14.4	14.0	1.0545	1.0260	48.0	40.3	−16
			Mean corpuscular volume 80–99 cu. μ			
4.8	15.2	1.0358	1.0260	20.0	14.0	−30
4.8	34.4	1.0370	1.0262	14.6	15.0	+ 2
7.5	15.2	1.0480	1.0290	29.0	28.0	− 3
9.0	19.1	1.0446	1.0240	33.3	28.2	−15
10.4	19.5	1.0480	1.0270	38.0	30.0	−21
10.5	15.2	1.0488	1.0298	36.0	28.3	−21
11.0	14.0	1.0505	1.0300	38.0	30.6	−19
13.0	16.0	1.0530	1.0280	44.5	36.3	−18
13.5	18.0	1.0540	1.0270	43.0	38.5	−10
13.5	15.8	1.0540	1.0280	41.0	37.7	− 8
13.8	19.3	1.0533	1.0270	45.0	37.5	−17
14.0	16.0	1.0540	1.0288	45.8	37.0	−19
14.2	23.3	1.0565	1.0295	47.5	40.0	−16
14.2	23.9	1.0550	1.0268	46.0	40.1	−13
14.2	16.0	1.0541	1.0268	45.0	38.9	−14
14.2	16.0	1.0550	1.0268	44.0	40.2	− 9
14.2	15.8	1.0570	1.0290	42.0	41.3	− 2
14.6	15.8	1.0551	1.0285	48.0	38.9	−19
14.8	16.0	1.0553	1.0259	49.0	41.3	−16
15.0	16.0	1.0565	1.0273	48.0	41.9	−13
15.0	16.0	1.0557	1.0257	48.0	42.0	−12
15.0	14.5	1.0560	1.0268	49.0	41.7	−15
15.1	16.0	1.0563	1.0268	47.2	42.0	−11
15.1	16.0	1.0579	1.0282	49.0	43.2	−12
15.4	15.8	1.0565	1.0260	48.0	43.0	−10
15.4	16.0	1.0560	1.0268	48.0	41.7	−13
15.6	15.8	1.0570	1.0266	48.0	43.3	−10
15.6	16.0	1.0598	1.0276	50.0	46.4	− 7
15.8	16.0	1.0573	1.0268	51.0	43.5	−15
16.0	15.8	1.0588	1.0259	48.0	46.4	− 3
16.0	15.8	1.0603	1.0280	51.0	47.0	− 8
16.4	15.8	1.0580	1.0269	50.0	44.4	−11
16.4	15.8	1.0589	1.0257	48.0	46.6	− 3
			Mean corpuscular volume 100–109 cu. μ			
3.7	14.1	1.0305	1.0230	11.5	10.1	−12
7.6	16.4	1.0412	1.0270	22.0	20.2	− 8
10.1	15.2	1.0489	1.0279	33.0	30.7	− 7
10.5	11.7	1.0488	1.0270	31.8	31.1	− 2
12.0	14.5	1.0522	1.0295	42.5	33.6	−21
12.0	14.5	1.0515	1.0300	41.0	32.1	−22
12.7	18.2	1.0539	1.0295	39.0	36.2	− 7
12.8	14.1	1.0530	1.0283	45.0	35.9	−20
13.3	18.2	1.0540	1.0290	40.0	36.8	− 8
13.8	15.5	1.0578	1.0308	41.5	40.7	− 2
14.0	16.1	1.0518	1.0250	44.9	37.2	−17
14.0	23.2	1.0530	1.0250	44.6	38.9	−13
16.5	35.3	1.0576	1.0270	49.0	43.5	−11

TABLE II—*continued*

Haemoglobin in g.%	Temp. in ° C.	Gb	Gp	CV found by haematocrit	CV calculated from sp. gr.	Difference in % CV (sp. gr.) / CV (haematocrit)
			Mean corpuscular volume 110–129 cu. μ			
2.2	16.0	1.0298	1.0256	7.5	5.9	−21
4.8	15.4	1.0400	1.0300	15.5	14.9	− 4
9.0	15.9	1.0442	1.0255	24.0	26.2	+14
9.0	18.2	1.0472	1.0275	30.8	23.4	− 8
9.6	27.5	1.0460	1.0260	32.0	28.2	−12
10.1	15.9	1.0508	1.0300	32.5	31.0	− 5
10.2	14.1	1.0466	1.0249	36.0	30.1	−16
10.7	17.8	1.0475	1.0222	36.5	33.9	− 7
11.2	15.3	1.0515	1.0305	37.0	31.5	−15
11.5	13.4	1.0508	1.0270	35.5	34.0	− 7
11.7	23.1	1.0522	1.0310	37.0	32.1	−13
12.6	15.3	1.0515	1.0249	40.5	36.8	−12
13.0	15.0	1.0510	1.0260	38.5	35.3	− 8
13.1	15.0	1.0515	1.0280	39.5	34.5	−13
14.8	15.1	1.0552	1.0280	45.0	39.5	−12
			Mean corpuscular volume above 130 cu. μ			
6.0	13.8	1.0370	1.0250	18.5	16.7	−10
6.4	16.5	1.0378	1.0230	20.0	20.0	± 0
7.0	12.8	1.0380	1.0220	22.0	21.4	− 3
7.4	23.4	1.0440	1.0288	23.5	22.3	− 5
9.2	23.9	1.0478	1.0278	28.6	28.9	+ 1
9.5	15.1	1.0478	1.0280	34.0	28.7	−16
9.8	14.5	1.0493	1.0283	35.0	30.6	−12
10.5	18.9	1.0490	1.0269	36.8	31.5	−14
11.4	14.5	1.0502	1.0270	40.0	33.1	−17
13.3	12.1	1.0540	1.0270	47.0	38.5	−18

There is in this series the usual association between high and low mean corpuscular volume and high and low mean corpuscular haemoglobin concentration. Therefore there is a tendency for the difference between the two CVs to rise the less haemoglobin the cells contain—that is, the more Gc becomes an expression of the gravity of the non-haemoglobin protein of the blood cells (see also Chart).

If the calculated CV values are multiplied with a factor of 1.1 more satisfactory agreement with the true CV is obtained.

In the Chart is shown the scatter of the mean corpuscular volume values based on CV haematocrit and on the CV (specific gravity) calculated according to the equation

$$(2) \quad CV = 110 \times \frac{Gb - Gp}{1.0970 - Gp}$$

where Gb and Gp are again the specific gravity of the oxalated blood and plasma respectively. The values obtained from blood having a mean corpuscular haemoglobin concentration of 30 per cent and above are entered as ●, those of bloods showing a mean corpuscular haemoglobin concentration below 30 per cent are shown as ▲. The diagonal line represents ideal agreement and all values should be found on it if the method were perfect. It will be seen that while the values are about equally scattered above and below the line of ideal agreement, divergencies in either direction are considerable. The figure also demonstrates that even on using the corrected equation a low mean corpuscular haemoglobin concentration tends to place the calculated results below the true value.

Table III shows the ranges in which mean corpuscular volumes based on equation (2) fall when compared with the true values. Seventy-two out of seventy-five cases fall within the limit set out in Table III. The three exceptions are the first two samples in Group 2 of Table II and the first sample in Group 4. All three samples have a haemoglobin content of below 5 g. per cent.

TABLE III

THE RANGE WITHIN WHICH MEAN CORPUSCULAR
VOLUMES FALL WHEN BASED ON CV VALUES
CALCULATED FROM EQUATION (2)

Number of cases	True mean corpuscular volumes (derived from haematocrit CV) in cu. μ	Range of mean corpuscular volumes calculated from specific gravities	Number of exceptions
4	70– 79	60– 75	
33	80– 99	75–105	2
13	100–109	85–110	
15	110–129	105–140	1
10	130–160	120–175	

It will be seen that in our hands the method of calculating CV and mean corpuscular volume from specific gravity was not accurate enough to differentiate normocytosis from macrocytosis and microcytosis respectively. Whereas the difference between the Ge in our samples from that given by the originators may be compensated by introducing the factor 1.1 into the original equation, no single correction can reduce the wide margin within which calculated results are obtained on either side of the true values.
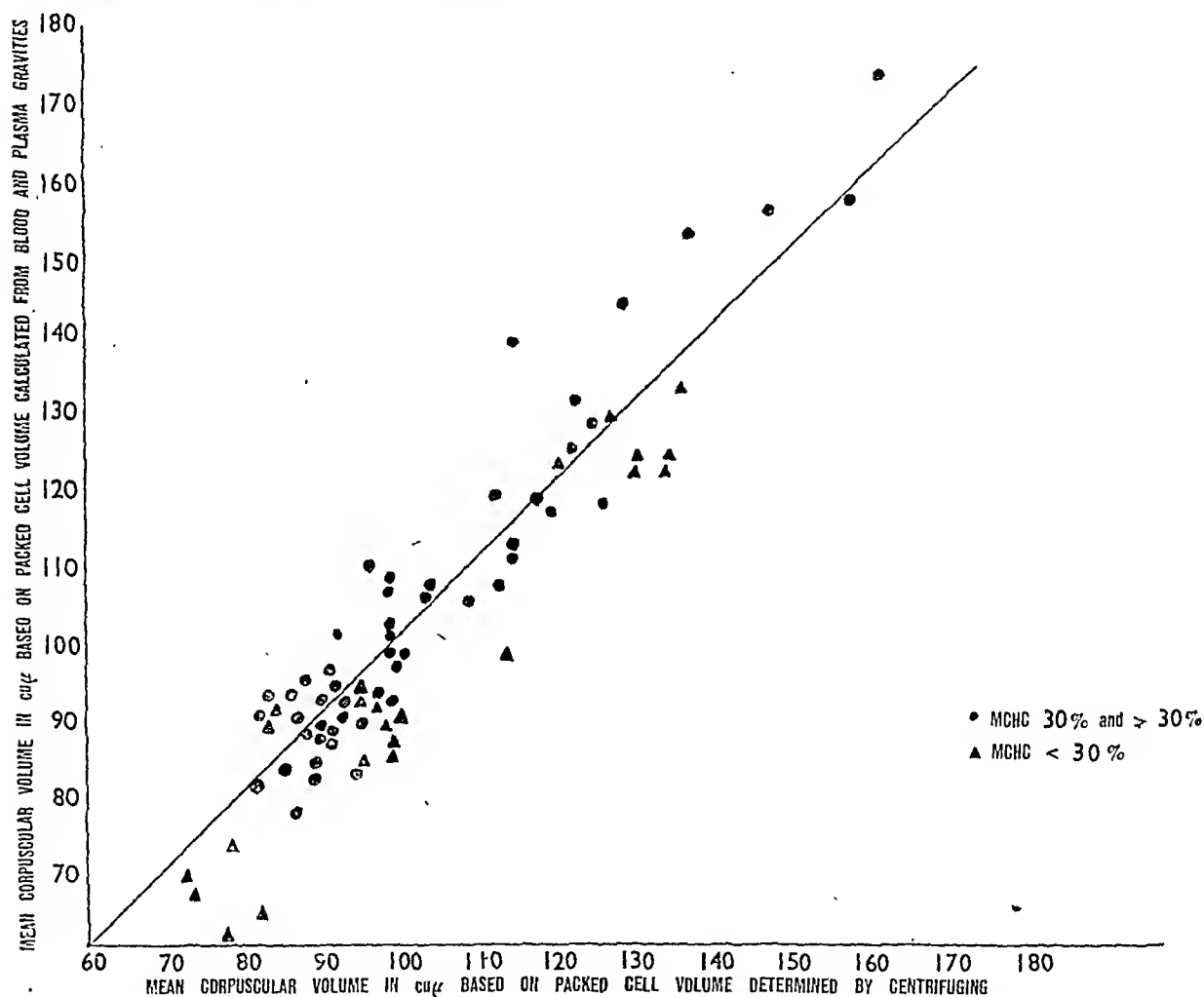
CHART.—The range within which agreement can be obtained between mean corpuscular volume derived from the haematocrit value and mean corpuscular volume based on a CV calculated from blood and plasma gravities. The influence of mean corpuscular haemoglobin concentration on this agreement is also shown.
Abscisse : Mean corpuscular volume in cu. μ derived from haematocrit values.
Ordinate : Mean corpuscular volume in cu. μ calculated from specific gravities.
● mean corpuscular haemoglobin concentration, 30 per cent and above.
▲ mean corpuscular haemoglobin concentration, below 30 per cent.

Summary

1. The haemoglobin content, the red cell concentration, the haematocrit value, the mean corpuscular volume, the mean corpuscular haemoglobin concentration, and the specific gravities of blood and plasma of seventy-five Indian soldiers were determined. Some of the subjects were hospital patients.

2. Nineteen per cent of the subjects were mildly, and 45 per cent more severely, anaemic. The mean corpuscular volume was below 100 cu. μ in one-half of the cases ; of the other half, one-third showed slight and two-thirds considerable macrocytosis.

3. The CV values (ml. of packed cells in 100 ml. of blood) found by centrifuging (CV haematocrit) were compared with those calculated from blood and plasma gravities (CV specific gravity).

4. The CV (specific gravity) was generally 10 per cent lower than the CV (haematocrit). The per cent differences increased with smaller mean corpuscular volume and lower mean corpuscular haemoglobin concentration.

5. It is suggested that the differences between the haematocrit CV and CV determined from specific gravities were due to the cell gravities in the samples being below 1.0970, the value given for normal cell gravity by previous authors, and that these cell gravities were the lower the more they depended on the non-haemoglobin protein of the cells.

6. The difference between the haematocrit and gravity cell-volumes could be fairly satisfactorily compensated by the introduction of the factor 1.1 into the equation from which CV specific gravity is calculated. However, mean corpuscular volumes based on CV specific gravity obtained in this way still diverged too widely on either side of the figures obtained from haematocrit values to make the calculation a useful method for clinical purposes.

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SUPPURATIVE MENINGITIS IN THE NEWBORN DUE TO COLIFORM BACILLI

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Although infrequent, suppurative meningitis in the first month of life is now more commonly recognized than formerly. Barron (1918), reviewing meningitis in infancy nearly thirty years ago, found only thirty-nine cases of the suppurative form reported in children under three months, nineteen of which were in the newborn. From this he concluded that suppurative meningitis is a rare disease at this age.

Cruickshank (1930), in a general survey of neonatal deaths, found that meningitis was responsible in thirty-three out of eight hundred cases, about 4 per cent. Flensborg (1942) was able to collect 140 reports from the literature of suppurative meningitis in infants under one month old. In his own series of 374 necropsies on newborn infants, he found three cases (0.8 per cent) of suppurative meningitis. Still more recently MacGregor (1946) has recorded the pathological findings in 618 cases of neonatal deaths. Infections of all kinds were responsible for 30.7 per cent of all deaths, and for 65.5 per cent of the 241 deaths which took place after the third day of life. In a personal communication (1947), she states that there were eight cases of suppurative meningitis during the five-year period of her review (1939–43), a percentage of 1.3 of the total deaths.

Six further cases of suppurative meningitis, all of them caused by coliform bacilli, are reported below.

Case Histories

CASE 1

The mother was a 10-para, aged 40 years. Labour was induced by rupture of the membranes on Oct. 18, 1946, and pains began early on the next day. She had no previous history of any infection of the birth canal or urinary tract. The patient was distressed during the first stage, and after twenty-one hours her pulse rate was 140 per minute and temperature 104° F. Foetal heart rate at this time was 150 per minute. The pyrexia subsided within three hours and did not recur.

The child was delivered after a first and second stage lasting thirty-eight hours. The infant was a male and weighed 7 lb. 14 oz. At that time it was cyanotic for a few minutes only. Following this nothing abnormal was noticed until he was put to the breast for the first time after birth, when 7 hours old. He would not suck, and there was a sudden onset of nystagmus and some twitching of the limbs. The child remained restless, vomited several times, and 13 hours after birth had the first of numerous convulsions. Death occurred 16 hours 40 minutes after delivery.

Post-mortem examination.—The body was that of a full-term child showing early evidence of dehydration. There were no external marks.

Respiratory system.—There was some mucus in the upper air passages. Sub-pleural petechial haemorrhages were seen over both lungs, and on section the organs themselves were atelectatic, showing only small areas of expansion.

Central nervous system.—The skull was normal. The dura did not bulge, and there was no tear of tentorium or falx. In the subarachnoid space there was a considerable amount of thick, greenish pus, particularly over the base of the brain, from where it tracked along the superficial cerebral veins to the superior surface of the hemispheres. There was no excess of cerebrospinal fluid and no internal hydrocephalus. On section the cerebral hemispheres, midbrain, cerebellum, and medulla were normal. Basal arteries, mastoid air-cells, and venous sinuses were normal. Examination of cardiovascular, alimentary, hepatic, genito-urinary, and endocrine systems revealed no abnormality.

Bacteriology.—Films of pus from the subarachnoid space showed many polymorphs and Gram-negative bacilli, some of which were intracellular. Cultures yielded a heavy growth of motile coliforms, giving the biochemical reactions of *Bact. coli communis.*

Histology.—The lung showed severe atelectasis of foetal type.

Various sections of the brain show acute purulent meningitis, with the majority of the cellular exudate made up of lymphocytes and other mononuclear cells, the number of polymorphs being small. Gram films show many Gram-negative bacilli in the exudate.

CASE 2

The mother was a 2-para, labour being surgically induced on Dec. 16. 1946, as it was several weeks overdue. The labour was normal, and the child, a male, was delivered on Dec. 18. His weight was 6 lb. 12 oz., and for the first few days his condition was satisfactory, although he did not feed well and was of a poor colour. On the sixth day there was a sudden increase of temperature to 102° F. and marked increase of respirations. On the following day he developed twitching movements of limbs. on several occasions amounting to convulsions. Neck rigidity was present, and Kernig's sign positive. A diagnosis of meningitis was made, but before any further steps could be taken the child lapsed into coma, dying on Dec. 26, 1946.

Post-mortem examination.—The body was that of a well developed male infant showing slight cyanosis but no other external abnormalities.

Central nervous system.—The skull was normal. The dura was normal, with no tears of falx or tentorium. The brain showed severe flattening of the convolutions. and a diffuse purulent exudate was present in the subarachnoid space. At the base of the brain this exudate was yellow and opaque. The basal arteries, venous sinuses, and mastoid air-cells were normal. Cerebral hemispheres, mid-brain, cerebellum, and medulla were normal on section. Apart from some patchy atelectasis of both lungs, examination of respiratory, alimentary, hepatic, genito-urinary, and endocrine systems revealed no abnormalities.

Histology.—Section of the brain showed a purulent exudate in the subarachnoid space of similar cytology to that in Case 1, with predominance of lymphocytes (Plate I*a*). Gram film of this section shows numerous Gram-negative bacilli, many of them intracellular and morphologically similar to *Bact. coli.* Unfortunately, no cultures were made from this specimen, but the finding of Gram-negative bacilli morphologically similar to *Bact. coli* and unaccompanied by any other bacteria suggests that they are the responsible organisms and that the condition is a coliform meningitis.

CASE 3

Mrs. P., a multipara, was delivered at her home of a full-term male child on Nov. 4, 1946, the birth weight being 6 lb. 2 oz. The antenatal period and labour had been normal. The infant was breast-fed for two days, but sucked badly and was put on to artificial feeds. The child was still feeding very poorly on Nov. 10, and as it appeared to be getting weaker was admitted to hospital. On examination he was drowsy and cold; there was some cyanosis and signs of early dehydration. He vomited soon after admission. On Nov. 12 no abnormalities could be detected on examination, except for a possible weakness of the right arm, and he was still not feeding well. On Nov. 14 he was feeding better. but the skin was dry and inelastic, suggesting dehydration. but the fontanelle was

not depressed. On Nov. 16 the pulse rate dropped from 150 on admission to below 100 per minute. Temperature still remained subnormal and the fontanelle tension was normal although general signs of dehydration were marked. There was, however, some separation of the cranial sutures. No neck rigidity was detected. On Nov. 17, 1946, the child was moribund and refusing all feeds. The fontanelle pressure was now increased, but there was still no neck rigidity. The pulse rate dropped to 64 per minute and death occurred on Nov. 18, 1946.

Post-mortem examination.—The body was that of a dehydrated male infant.

Respiratory system.—The upper air passages showed acute inflammatory changes, and the pleural cavities contained 15 c.cm. of straw-coloured effusion. On section the bases of both lungs showed a patchy pneumonic consolidation, and there were some areas of lung which had not fully expanded.

Central nervous system.—The skull was normal. The dura was bulging, and there was marked engorgement of the leptomeninges, with a thin sero-purulent exudate in the subarachnoid space, particularly marked at the base of the brain. The venous sinuses, basal arteries, and mastoid air-cells were normal. The brain was oedematous on section, but otherwise normal throughout.

Examination of cardiovascular, alimentary, hepatic, genito-urinary, and endocrine systems revealed no abnormality.

Bacteriology.—Films made from the subarachnoid space at necropsy showed numerous Gram-negative bacilli and pus cells. Cultures yielded heavy growths of coliforms giving the biochemical reactions of *Bact. coli communis.*

Histology.—Sections of the brain showed an acute purulent leptomeningitis with a considerable amount of haemorrhage amongst the exudate, and a cytology predominantly lymphocytic in character.

Sections of lung showed acute bronchopneumonic congestion and some collapse.

Cases 4 and 5 are siblings and two of quadruplets. As the hospital was bombed soon after their death the clinical notes were destroyed. and the histories are, therefore, lacking in detail.

CASE 4

L. was the first delivered of quadruplets born prematurely on June 3, 1944, with a birth weight of 3 lb. 9 oz. She appeared well until the seventh day, when there was a sudden rise of temperature to 102° F. with the development of gradual abdominal distension and jaundice during the succeeding days. Cerebral screaming attacks also occurred. The blood count showed a mild leucocytosis of 25,000 cells per c.mm. During the performance of the differential count, which was normal. a macrophage (Plate I*b*) containing what appeared to be ingested bacteria was seen;

this suggested the presence of septicaemia, as did the continued fever and gradually increasing jaundice before death on June 19, 1944, at the age of 16 days.

Post-mortem examination.—The body was that of a small and premature female infant. The skin and all internal structures were deeply jaundiced.

Central nervous system.—The skull and dura were normal. Acute inflammatory changes were present in the leptomeninges with thin yellowish pus over the vertex and superior aspect of the cerebellum. There was also exudate over most of the basal cisterns. The cerebrospinal fluid in the ventricles was frankly purulent. The basal arteries, venous sinuses, and mastoid air-cells were normal. Sections of the brain were normal throughout. Examination of all the other systems revealed no abnormality.

Bacteriology.—Swabs taken from the brain and ventricles at post mortem showed large numbers of pus cells and Gram-negative bacilli in the stained films. Cultures yielded a pure growth of motile coliforms.

Histology.—Sections of the cerebral cortex showed acute inflammatory changes, but unfortunately the meninges from this specimen were lost.

Sections of the liver showed exudation of bile pigment, congestion, and fatty change.

Case 5

D. was born prematurely on June 3, 1944, the second to be delivered with a birth weight of 3 lb. 13 oz. His condition was satisfactory for the first few days, but he began to feed poorly at about the fourth day and when six days old developed a sudden rise of temperature. A day or so later there was onset of cerebral screaming attacks with gross abdominal distension. Treatment was begun with sulphathiazole by mouth, but there was gradual deterioration until death on June 14, 1944, when the child was 11 days old.

Post-mortem examination.—The body was that of a small premature infant showing some abdominal distension and generalized jaundice of the skin and internal structures.

Central nervous system.—The skull and dura were normal. The subarachnoid space contained a purulent exudate that was particularly thick over the vertex, superior aspect of the cerebellum, and tips of the temporal lobes. The cerebrospinal fluid was cloudy but not frankly purulent. Venous sinuses and basal arteries were normal. The right mastoid air-cells contained a little pus, but those on the left were normal. Examination of the cardiovascular, respiratory, alimentary, hepatic, genito-urinary, and endocrine systems revealed only scattered sub-serosal petechial haemorrhages.

Bacteriology.—Films from ventricular fluid, right mastoid, and subarachnoid space showed numerous pus cells and many Gram-negative bacilli. Cultures all yielded a heavy growth of *Bact. coli communis.* Culture from the left mastoid yielded only a few colonies of *Staph. albus.*

Histology.—There was acute purulent leptomeningitis of the brain, the underlying brain showing some congestion.

Sections of liver showed fatty change, exudation of bile pigment, and some areas of focal necrosis.

Case 6

The male infant was born by normal labour on April 29, 1947. The child appeared well until it was 3 days old, when it suddenly became grey, with difficult and distressed breathing. The birth weight was 8 lb. 3 oz., and at the onset of symptoms had fallen to 8 lb. 1 oz. Later the same day the temperature suddenly rose to 103° F. with grunting respiration. The infant had repeated atttacks of spasm of the hand muscles, similar to tetani. There was no neck rigidity and the pulse was then uncountable. He was given oxygen and chloral, the temperature fell to 102.4° F. at 6 a.m. on May 3, but rose suddenly to 105.6° F. at 10 a.m. a quarter of an hour before death.

Post-mortem examination.—The body was that of a well-developed male infant. There was moderate cyanosis of mouth and finger tips, but no dehydration or jaundice.

Respiratory system.—The upper air passages contained a large quantity of purulent material and both lungs showed on section a generalized bronchopneumonia. The pleural surfaces were normal.

Central nervous system.—The skull was normal. The dura was normal, with no tear of falx or tentorium. A greenish exudate which had a faecal odour was present in the subarachnoid space. It covered the base of the brain and tracked to the vertex. There was no excess of fluid in the ventricular system. There were no flattening of the convolutions, and the brain was normal throughout on section. The basal arteries, venous sinuses, and mastoid air-cells were normal.

Bacteriology.—Swabs of the subarachnoid space showed numerous Gram-negative bacilli and pus cells. Cultures yielded a heavy and pure growth of *Bact. coli communis.* This organism was not inhibited by 75 units per ml. of penicillin but was by 100 units of penicillin. Swabs from lung showed numerous Gram-negative cocci and some Gram-positive bacilli. Cultures yielded a good growth of *Bact. coli communis* and a few colonies of *Staph. albus.* The coliform in this culture showed similar results to penicillin sensitivity tests as did that isolated from the brain.

Histology.—Sections of the brain showed a purulent leptomeningitis.

Discussion

Incidence.—The age at death in Cruickshank's (1930) series of suppurative meningitis cases varied from 2 to 17 days, 88 per cent of the infants dying between the fifth and thirteenth day.

In Levinson's (1945) group of cases there were eight under one month, and five of these were in infants 14 days old or less; the youngest was 3
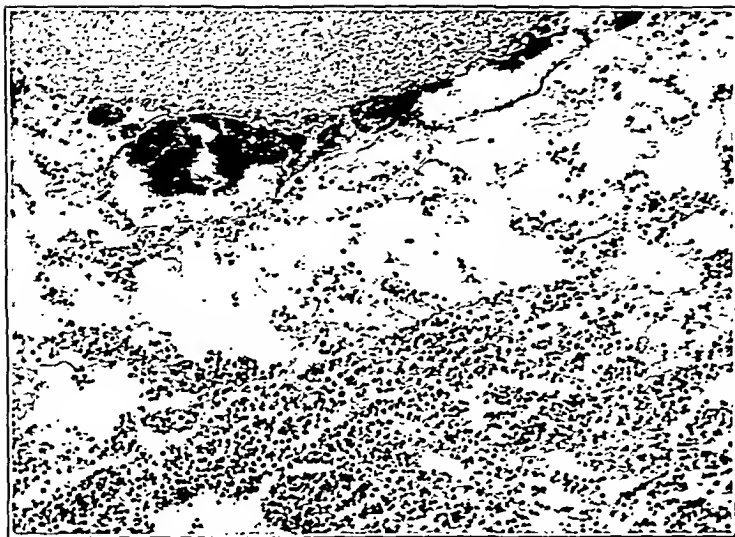
(a).—Section of the brain in Case 2, showing a purulent exudate in the subarachnoid space, with predominance of lymphocytes.

(b).—Macrophage containing what appear to be ingested bacteria, Case 4.

PLATE I

L

days, and the eldest 21 days. MacGregor's cases were all under 3 weeks, the ages at death being 16, 9, 9, 7, 5, and 4 days in the uncomplicated cases. In two others death occurred at 10 days and 6 hours respectively, following an infection of a meningocele in spina bifida.

In the present series, all were cases of primary meningitis except possibly Case 4, where infection of the mastoid air-cells may have preceded the meningitis. All deaths occurred under 3 weeks of age, at 17 hours, 8, 14, 11, 16, and 4 days. Case 1 is of particular interest, as there does not appear to be any previous record in the literature of a death from primary suppurative meningitis occurring less than 24 hours after delivery. In this incidence infection most probably occurred *in utero* at the time labour was induced.

Both Craig (1936) and Cruickshank state that infants developing meningitis are often premature. Two of the present cases were born prematurely. They were from a family of quadruplets, and it is well recognized that such infants born in multiple pregnancies possess a lower resistance to infection than those born at full term and of a single pregnancy. Of the two remaining children in this family, one, who had a rise of temperature during the second week of life, died of hydrocephalus at the age of 6 months. This condition may well have been secondary to unrecognized meningitis in the neonatal period, although no necropsy was performed to confirm it. The fourth child is alive and well and now 3 years old.

Symptomatology and diagnosis.—During the newborn period and in early infancy meningitis presents few, if any, symptoms which are referable to the local condition. The onset may be sudden or gradual. The temperature frequently remains normal, as was the case in 25 per cent of Levinson's series. However, a sudden rise of temperature may be an early sign, and this was observed in four of the six cases here reported. Other early signs are failure to feed and restlessness (three cases) and twitching of limbs or muscular spasms (three cases). Neck rigidity is not common and was only observed in Case 2. Convulsions frequently occur before death, and a cephalic cry may develop. Bulging of the fontanelle was observed in only one case, although Levinson states that it is nearly always present. All these signs are suggestive of an intracranial lesion or at any rate cerebral anoxia, which may also be produced by atelectasis, neonatal pneumonia, septicaemia, and neonatal tetani. Under these circumstances it is important that immediate examination of the cerebrospinal fluid

following lumbar puncture should be performed. This will confirm or deny any suspicion of the possibility of an intracranial lesion being present. It will also enable a differential diagnosis to be made as between meningitis and intracranial subdural haemorrhage.

In addition, Fothergill and Sweet (1933), having isolated the organism from the blood as well as the cerebrospinal fluid in 88 per cent of cases occurring in infants between 1 and 5 weeks old, state that bacteriacmia is very common in this type of meningitis. In this series the occurrence of bacilli in the blood was demonstrated in Case 4, where a macrophage containing ingested bacteria was seen in blood film (Plate 1*b*).

Bacteriology.—It is probable that in all the six cases outlined above the responsible organism was *Bact. coli*, and a certain bacteriological diagnosis was made in five of them, as it is extremely unlikely that the presence of coliforms isolated was due to post-mortem infection of tissues. There have been two reviews where the bacteriology of neonatal meningitis has been classified—that of Cruickshank (1930) and a later one of Flensborg (1942), which includes all cases in the literature at that time. To these should be added MacGregor's cases (see Table).

TABLE

ANALYSIS OF CASES OF NEONATAL MENINGITIS IN THE LITERATURE

	Cruickshank (1930)	Flensborg (1942)	MacGregor (1946)
Bact. coli ..	11	68	6
Staph.	1	9	—
Strep. ..	7	23	—
Staph. + Strep.	—	7	—
Pneumococcus	3	12	1
H. influenzae ..	—	4	—
N. catarrhalis ..	—	1	—
Gonococcus ..	—	1	—
Mixed flora ..	—	4	—
Ps. pyocyaneus	1	—	—

In contrast Neal (1926) reviewed fifty cases of meningitis occurring in children over three weeks and under three months of age. The incidence of the various organisms in thirty-five cases where bacteriological diagnosis was made was as follows:

N. meningococcus :	24 cases
M. Tuberculosis :	5 cases
Pneumococcus :	3 cases
Bact. coli :	3 cases

From the findings in all these reviews, coliforms are clearly the commonest pathogen in neonatal

meningitis. However, its incidence decreases with age and it is rarely found over two years, when meningococcal meningitis is reaching its maximal incidence. Although the most susceptible age period for this infection is given from 0 to 5 years (Compton, 1918), it is unusual in the first three months of life (Topley and Wilson, 1946). A possible reason for its low incidence may be found in the possession of a congenital immunity which lasts only for a few months. The restricted age incidence of coliform meningitis is not easily explained, but some light may be thrown on this point by a study of specific agglutinins present, in the sera of children at birth, to these and similar organisms.

Ravid (1935) says that "in the blood of foetuses and the newborn agglutinins to *B. coli* are absent," but he presents no evidence to substantiate this statement. Flensborg in his review also states that the newborn lack normal coli agglutinins present in adults.

Recently Wright (1947) has studied the transmission of coli agglutinins across the placenta, using seven different suspensions of coliforms obtained from " pathological " lesions. She found : (1) that the titres of both " O " and " H " agglutinins in the mother's serum at the time of delivery corresponded closely to that in the cord blood of the infant ; (2) that of 55 mothers and babies examined, practically none possessed agglutinins to all the seven suspensions, and one or two no agglutinins at all. Most of the subjects possessed recognizable agglutinins to about half the suspensions used.

From her results it seems likely that nearly every infant is vulnerable to any strain of coliform for which little or no agglutinins are present, and that chance contacts with such strains may result in infection. This susceptibility, together with the well recognized feebleness of antibody production during the early months of life, may explain the high incidence of coliform meningitis at this age.

Pathology.—The pathological changes in these cases differ little from those seen in other types of suppurative meningitis except for the predominance of lymphocytes and mononuclear cells in the exudate. In most of the infants the meningitis probably followed bacteriaemia. The portal of entry of the responsible organisms is, however, uncertain clinically, and the post-mortem findings give no further assistance in locating the site of primary infection.

Treatment.—Barrett and others (1942) say that the mortality without chemotherapy is 80 per cent.

and none of the cases in the present series recovered. They review six cases in which sulphonamides were used. There was only one death in the acute phase, although at least one other appeared to have died later from hydrocephalus. They say that sulphathiazole was then the drug of choice.

Since then, Pearlman and Bell (1944) have recorded a death in neonatal meningitis following adequate treatment with various sulphonamides in a newborn child, and Kohlbry (1942) a recovery in a male child aged 7 days treated with sulphapyridine. In an older patient Alexander (1946) treated successfully a coli meningitis following extensive fractures and osteitis of the right tibia (male, aged 19 years), with streptomycin.

As the organism in Case 6 was sensitive to 100 units per ml. of penicillin, yet another possible line of treatment was opened up by giving a large dose of the drug intrathecally.

However, the number of treated cases is still very small and it is hoped that, with earlier diagnosis in the future, these methods of chemotherapy may be more fully tested.

Summary

The literature on neonatal meningitis is reviewed and a series of six fatal cases described.

The aetiology, symptomatology, and bacteriology are discussed, views being presented to explain the predominance of coliforms as the responsible pathogen. Possible lines of treatment are discussed.

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(a).—Section through the spinal meninges of a ease of tuberculous meningitis, showing the fibrin masses in the upper half of the pieture.

(b).—Areas of clearance of test organism produced by streptomyein plus heparin (C) and streptomycin alone (B). No clearance round heparin alone (A).
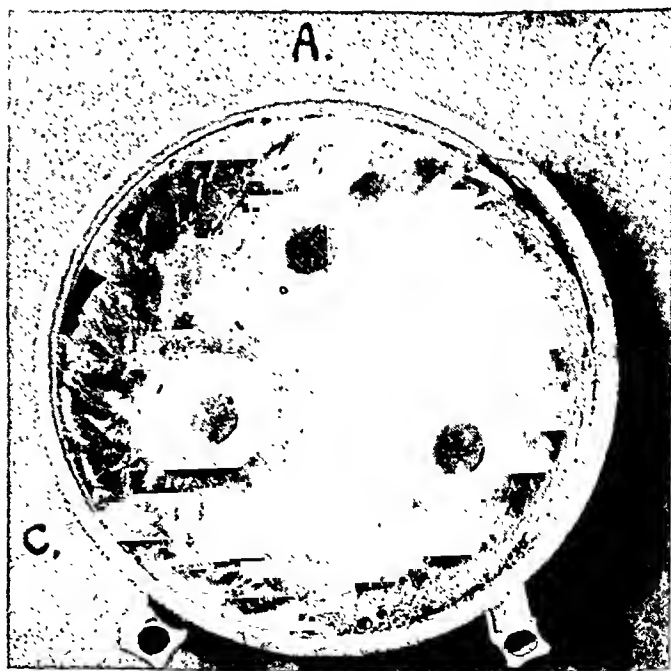
PLATE II

THE USE OF INTRATHECAL HEPARIN IN CONJUNCTION WITH STREPTOMYCIN IN THE TREATMENT OF TUBERCULOUS MENINGITIS

PRELIMINARY REPORT

BY

CHARLES A. ST. HILL, CLIFFORD RILEY, AND J. HAMILTON GIFFORD

From the Royal Southern Hospital and the Fazakerley Sanatorium, Liverpool

One of the commonest causes of failure in the treatment of tuberculous meningitis by streptomycin is the formation of intracranial and intraspinal adhesions with resultant hydrocephalus. These adhesions are produced partly as a result of the fibroblastic reaction that occurs around tuberculous foci, but their inception and growth may well be stimulated by the deposition of fibrin from the cerebrospinal fluid which is rich in fibrinogen in this condition. The photomicrograph (Plate IIa) illustrates that fibrin is, in fact, laid down in this condition. This was taken from the spinal cord of a child who had died from tuberculous meningitis after several weeks of treatment by streptomycin, and the masses of fibrin in the upper part of the picture can be clearly seen. If fibrin deposition is of importance in the formation of tuberculous adhesions, therefore, it might reasonably be hoped that the prognosis would improve if this could be prevented.

The obvious drug for this purpose is heparin. We decided for this reason to investigate the possibility of introducing this substance into the cerebrospinal fluid with streptomycin, and to attempt to assess its value in the treatment of tuberculous meningitis. Our preliminary findings on the interaction of streptomycin and heparin appeared to be of sufficient interest to warrant this preliminary communication. The investigations so far completed, and the conclusions derived therefrom, are given below.

Effects of Admixture of Heparin and Streptomycin Solutions

When a solution of heparin ("liquemin," or "heparin Boots," or "heparin Evans") was added to one of commercial streptomycin (hydrochloride or calcium complex), a precipitate was slowly formed. The nature of this precipitate has not

M

yet been fully investigated, but certain properties have been noted. Examination of the supernatant fluid after centrifugalization revealed that there was no diminution of the streptomycin content during this reaction. Streptomycin assays were carried out both by serial dilutions in fluid media and cup plate methods.

The results of one of the latter methods of assay are shown in Plate IIb. In this experiment, one drop of streptomycin solution of strength 25 units per ml. was added in the cup C to one drop of heparin solution of strength 1,000 units per ml., thus ensuring that a very large excess of the latter was present. The photograph shows that there was no diminution of the area of clearance of the test organism by this mixture as compared with the control cup B, into which was placed one drop of the same streptomycin solution and one drop of saline. Into cup A was placed one drop of a 1,000 unit per ml. heparin solution in order to demonstrate that it has no bacteriostatic powers.

Solutions of known strengths of heparin and streptomycin were again mixed and, after centrifugalization, the heparin contents of the supernatant fluid and of the deposit were estimated.

In the earlier stages of the work this heparin content was assayed on the basis of its delaying powers upon the deposition of fibrin from human plasma in the presence of excess thrombokinase and adequate calcium. This method is based on the assumption that heparin exerts its influence by the inactivation of thrombin and prothrombin (Best and Taylor, 1945). As McIntosh (1941) has pointed out, however, this method tends to give inconsistent results so that it was later replaced by a slight modification of the toluidine blue method of Trethewic and Melvin (1945). We have compared the colours of the unknown heparin toluidine blue mixture with that of the standard in a colorimeter instead of in comparator tubes as described by them. This method is quicker and gives consistent results but, of course, measures heparin in colour units.

These assays showed that:

(*a*).—If the amount of heparin in solution (in mg.) was less than approximately two-thirds of the amount of streptomycin (also in mg.) to which it was added, then no heparin was present in the supernatant fluid after removal of the precipitate. This was true whether the heparin was assayed by the toluidine blue method or by its anticoagulatory effect. This applies to the commercial preparations of heparin used by us, which contained between 90 and 110 units of heparin per mg.

(*b*).—If more heparin was then added to the mixture a further precipitation occurred, but active heparin began to appear in the supernatant fluid, so that by adding sufficient excess of heparin, concentrations of the order of 1,000 units per ml. or higher could be obtained free in the clear supernatant fluid in the presence of streptomycin. Even when these concentrations of heparin had been obtained, the addition of further heparin to the mixture still produced a precipitate. The addition of more streptomycin, on the other hand, did not cause a precipitate. Thus it was possible to prepare a mixture of heparin and streptomycin in clear solution and high concentration which could be introduced into the theca even though previously injected streptomycin was still present, without the

danger that an intrathecal precipitation might occur.

(*c*).—The precipitate, which consisted of a mixture of a white powdery material and a black tarry substance, contained active heparin as revealed both by the toluidine blue method and its anticoagulatory effect. As no streptomycin was lost from solution, there was presumably no streptomycin in the precipitate. Thus the precipitate must have been produced by a reaction between heparin and some impurity in the streptomycin.

(*d*).—All the heparin lost from the supernatant fluid was found to be present in the precipitate.

(*e*).—The precipitate showed slight solubility when warmed.

Effect of Introducing Heparin into the Theca of a Patient receiving Intrathecal Streptomycin

This was only tried on one patient, owing to the adverse reaction obtained. One ml. of a 1,000 unit per ml. solution of heparin was introduced into the theca daily, immediately after the introduction of streptomycin, and at the end of the twenty-four-hour period the streptomycin and heparin contents of the cerebrospinal fluid were assayed. The changes in the cerebrospinal fluid and the reaction of the patient are given in the composite graph

Date	Oct. 11	18	25	31	Nov. 3	12	18	21
Weight (lb.)	26·7	26·5	28·5	28·5	27·75	26·5	25·0	25·25
E.S.R. (1 Hr. Wintrobe)	34			34	52	34		
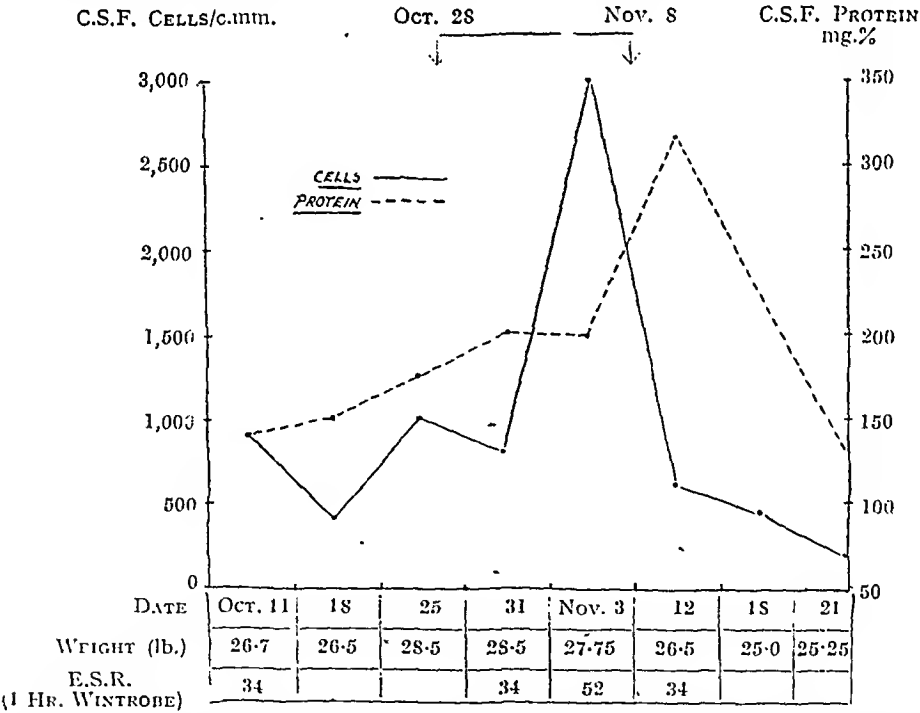
Fig. 1.—Changes in the cerebrospinal fluid, weight, and erythrocyte sedimentation rate during a course of daily intrathecal injections of heparin and streptomycin given separately. From Oct. 28 to Nov. 8 the patient had been receiving intrathecal and intramuscular streptomycin.

(Fig. 1). As can be seen from this, the white cells and protein contents of the cerebrospinal fluid rose sharply to 3,000 per c.mm. and 320 mg. per 100 ml. respectively. Coinciding with this, the patient began to show neck rigidity and incontinence with increased irritability and anorexia, whilst his weight, which had previously been increasing, started to decrease. On several occasions he showed evidence of experiencing pain immediately after the heparin injections, but it was impossible to confirm this as he was not co-operative.

This single case suggested that this method of giving heparin was not practicable. A combined solution of heparin and streptomycin was therefore prepared by adding 80,000 units of heparin in 10 ml. of saline to 1,000,000 units (1 g.) of streptomycin in an equal volume of saline. The mixture was allowed to stand in the refrigerator overnight to allow the maximum deposition of precipitate. The clear supernatant fluid was then decanted. The streptomycin content of this clear fluid was 50,000 units per ml., whilst the heparin content was found to be approximately 1,000 units per ml. This was injected in 2.0 ml. doses intrathecally into the same patient who had previously reacted adversely to the separate injections of streptomycin and heparin. The graph (Fig. 2) shows that the reaction produced was slight. We were thus encouraged to repeat the process in two other patients who had just been admitted and who had received no streptomycin previously. The changes in the cerebrospinal fluid of one of these are shown in Fig. 3 and, as can be seen, there was very little adverse reaction. The dose of free heparin was later increased to 5,000 units per dose in order to produce a residual concentration of between 1 and 2 units of heparin per ml. of cerebrospinal fluid at the end of the twenty-four-hour period.

Effect upon Heparin of the Streptomycin of the Cerebrospinal Fluid, following Purely Intramuscular Injections of this Latter Substance

It would appear probable that the precipitate formed by the admixture of solutions of heparin
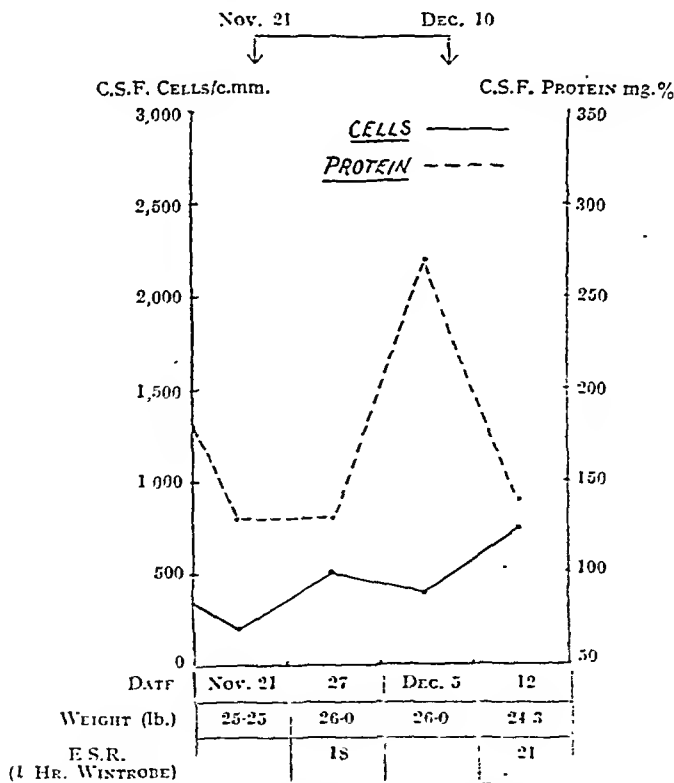
FIG. 2.—Cerebrospinal fluid changes during a course of daily injections of heparin–streptomycin mixture from Nov. 21 to Dec. 10. Case 1.
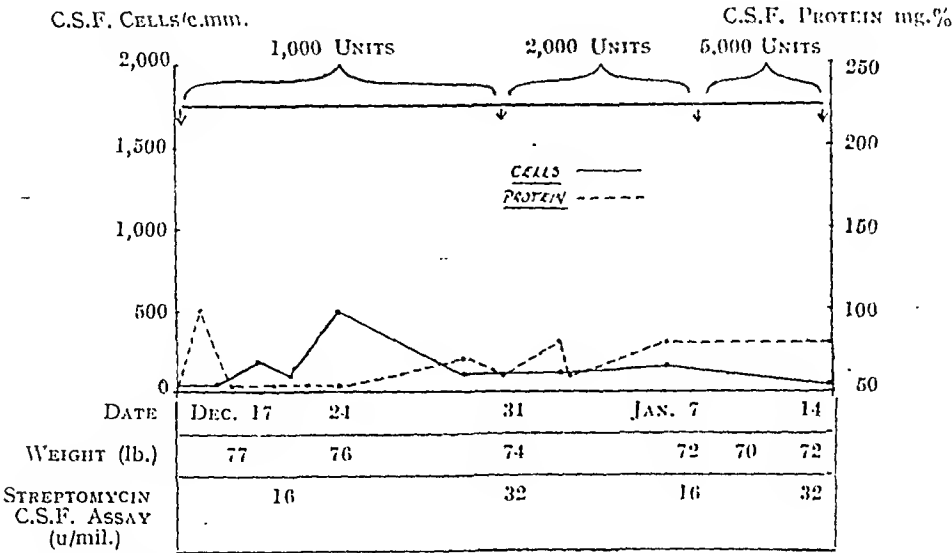
FIG. 3.—Cerebrospinal fluid changes during the daily administration of heparin–streptomycin mixture containing a higher concentration of heparin than in Case 1.

and streptomycin is caused by impurities in the streptomycin. It was decided, therefore, to determine whether streptomycin that has been given intramuscularly and has reached the cerebrospinal fluid via the blood stream has a similar effect upon heparin solutions, that is, the production of a precipitate. Specimens of cerebrospinal fluid were obtained from patients receiving streptomycin by the intramuscular route only. These were withdrawn about one hour after the intramuscular streptomycin had been given, so that the concentration of the drug should be at a maximum. Equal quantities of this and of known strengths of heparin solutions were mixed, allowed to stand for.

twelve hours, centrifugalized, and the surviving heparin assayed. It was found that in all cases a small proportion of the heparin was lost, but that this was not related to the amount of streptomycin present in the cerebrospinal fluid samples. It may well be that the losses that occurred were due to fixation of heparin by fibrin precursors of the cerebrospinal fluid.

Effect of Injecting Heparin into the Theca of Patients receiving Intramuscular Streptomycin Only

The evidence derived from the preceding experiment suggested that no adverse reaction would
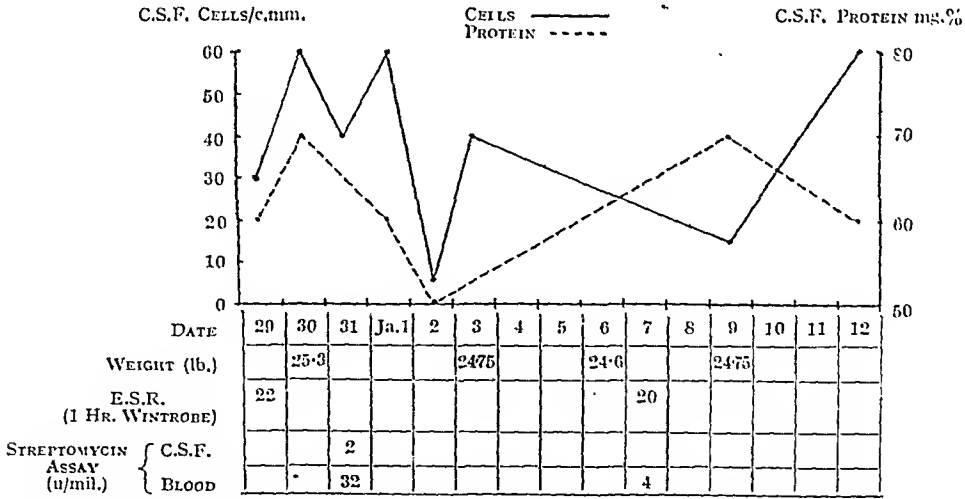
FIG. 4.—Changes in the cerebrospinal fluid during a course of daily intrathecal injections of heparin in a patient on intramuscular streptomycin.

follow the intrathecal administration of heparin to a patient who was receiving streptomycin by the intramuscular route only. Heparin was introduced, therefore, in 2,000-unit doses daily into the theca of a patient receiving this form of therapy, and changes in his cerebrospinal fluid and clinical condition were noted. The graph (Fig. 4) shows that no significant adverse reaction occurred.

Conclusions

1. The admixture of streptomycin and heparin causes the appearance of a precipitate which removes a large proportion of the heparin from solution, but has no effect on the streptomycin.

2. The occurrence of this reaction in the spinal theca produces an adverse effect on the patient.

3. A mixture of streptomycin and heparin in clear solution can be prepared which can be introduced with safety into the theca and gives comparable streptomycin assays with those found if streptomycin is given alone.

4. When the patient is being given intramuscular streptomycin only, heparin can be introduced into the theca without causing a reaction.

By this means it is possible to inject heparin regularly into the thecae of patients suffering from tuberculous meningitis under streptomycin treatment in an attempt to delay or inhibit the formation of adhesions. The effect of this on prognosis has yet to be investigated.

Addendum

Since sending this article to press we have been receiving samples of very pure heparin from the Evans Biological Institute, Runcorn. Very little precipitation results from the admixture of solutions of this heparin and streptomycin, and the supernatant fluid contains a very high concentration of heparin. This has resulted in a saving of heparin, but accentuates the need for assaying the heparin content of all heparin/streptomycin mixtures before use.

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A STANDARDIZED MODIFICATION OF THE LAUGHLEN TEST FOR SYPHILIS

BY

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Laughlen published the first account of his test for syphilis in 1935.

The antigen was made thus: to 1 ml. of Kahn antigen was added a "small quantity" of a fat stain (Scharlach R or Sudan III) and four drops of tinct. benzoini co., B.P. The mixture was warmed in a water-bath at 50° C., and 10 ml. of 1.5 per cent saline, also warmed to 50° C., was added. This pink, milky-looking fluid was "sensitized" by the addition of varying amounts of 9 per cent salt solution. For the test one drop of this sensitized antigen was mixed on a slide with one drop of the serum under examination and gently rocked for 10 minutes; precipitation occurring within this time indicated a positive result. Several modifications of this technique have been described by various workers.

The Present Study

The present study was undertaken with a view to finding a simple method of making a reliable Laughlen antigen which was capable of being scientifically reproduced in a standard manner. The components of the antigen were first considered individually.

Kahn antigen.—Experiments with Kahn antigens from several sources showed that the one produced by Burroughs Wellcome and Co. was highly sensitive—it was used in all subsequent work and proved satisfactory.

Stains.—The "knifepointfuls" and "small quantities" referred to in some versions of the Laughlen test are too vague for accurate work. It was found that a simple method of overcoming the difficulty of the very small amount of stain required was to use a 3 per cent alcoholic solution made as follows: to 100 ml. of absolute ethyl alcohol in a 250 ml. flask is added 3 g. of the stain powder. The flask, stoppered tightly with cotton wool covered with tinfoil, is shaken for 5 minutes in a Kahn "shaker" and placed in an incubator at 37° C. for 30 minutes. The resulting solution is dark red but contains a thick deposit of insoluble material, which is removed by filtering twice through Whatman's No. 1 paper. The volume is made up to 100 ml. with absolute alcohol. By using this solution the stain may now be accurately measured, 0.1 ml. being added to 1 ml. of Kahn antigen. This amount of stain solution has no adverse effect on the final Laughlen antigen. Of several fat stains tried, Scharlach R was found to be the best.

The diluting saline.—Better results were obtained with the Laughlen test when the diluting saline was buffered, with optimum value at pH 6.6. The buffer-saline consists of 9 g. NaCl added to 1 litre of $M/15$ Sorensen's phosphates at pH 6.6. Pyrex glass is used throughout, as a precaution against increasing alkalinity. The figure mentioned above, 0.9 per cent, has been found to be the optimum NaCl concentration at pH 6.6. With buffer-saline it is to be noted that alteration of the salt content does not produce the same wide variations in antigen sensitivity as is found with ordinary saline.

The benzoin.—A 1-ml. pipette delivers 0.3 ml. (the optimum quantity) tinct. benzoini co. more accurately than "drops."

PREPARATION OF ANTIGEN

Into a Pyrex test-tube (6 in. × 5/8 in.) pipette is put 10 ml. of buffer-saline. The tube is placed in a water-bath at 50° C. and left while the other components of the antigen are prepared. To 1 ml. of Kahn antigen (Burroughs Wellcome and Co.) in a similar tube is added 0.1 ml. of the 3 per cent stain solution and 0.3 ml. tinct. benzoini co., and this is placed in the water-bath for 2 minutes. Both tubes are removed and the contents mixed by pouring the saline into the antigen, back and forwards four times. The resulting pink liquid is the modified Laughlen antigen. Sedimentation, which occurs within a few hours, does not impair the qualities of the antigen: before use the colloidal appearance is restored by inverting the container. The sensitivity of the completed antigen is low when first made, but rises to its maximum within the next 30 to 36 hours, thereafter remaining constant for about four months. It should, therefore, be laid aside for about 36 hours before testing. Further sensitization by strong saline is unnecessary. The antigen is stored at room temperature.

THE APPARATUS

The mechanical mixer (Plate IIIa) consists of a circular porcelain plate, marked in 3/4-in. diameter

circles with black enamel, mounted for rotation at 20° to the horizontal on an adapted gramophone motor.

Two Dreyer pipettes are needed, one for the antigen and one for sera. They are each calibrated to deliver 0.05 ml. serum per drop.

THE TEST

The sera are inactivated as usual. The need for inactivation has been questioned by several workers using the Laughlen test, but experience with the present modification has shown it to be necessary (Table I).

Method.—Tube racks are designed to take 12 specimens per row; it is convenient to have two-row racks, each row of which is given a reference letter (A, B, etc.) which is marked on the plate as shown in Plate IIIa. Using the appropriate pipette, rinsed out once with saline between specimens, one drop (0.05 ml.) of each serum is placed in its respective circle, working from left to right, and starting with the outer six circles. The next row is pipetted on to the other half of the plate. Into each circle is now added one drop of antigen, which is mixed by inversion just before use. The motor of the " mixer " is wound up and the plate placed on the turntable, which is set going at the standard speed of 18 to 20 revolutions per minute for 10 minutes. This " standard speed " is empirical and is simply that which makes for smooth mixing of the contents of the circles without causing overflowing. (The speed control of the motor may need adjustment to obtain this slow rate.) The mixtures of antigen and serum should fill each circle and flow round smoothly inside the margins, but if any remains as a blob of liquid the motor should be stopped momentarily and with a piece of thin wire the fluid may be spread out gently to fill the circle's area. At the end of the 10 minutes the plate is removed from the turntable and the twenty-four tests examined by means of a ×6 hand lens. The following results may be found:

Negative.—Appearance uniformly colloidal. No precipitate visible even with the lens.

Doubtful (÷).—A very faint precipitate visible with the lens. Of no *diagnostic* importance.

Positive (÷÷).—A definite, fine precipitate, visible to the naked eye. Does not indicate syphilis *per se*, and requires confirmation by Wassermann and/or Kahn reactions.

Positive (÷÷÷).—A heavy precipitate. Indicates syphilis in 95 per cent of cases.

Positive (÷÷÷÷).—A very heavy precipitate, concentrated into roughly twelve " clumps," always indicates syphilis and is invariably accompanied by a ÷÷ W.R. and a ÷÷, ÷÷÷, or ÷÷÷÷ Kahn.

The various degrees of positive precipitation are shown diagrammatically in Plate IIIb.

TABLE I

RESULTS OBTAINED WITH 75 SERA BEFORE AND AFTER INACTIVATION

			Modified Laughlen				
		Before inactivation			After inactivation		
W.R.	Kahn	÷÷, ÷÷÷ or ÷÷÷÷	÷	—	÷÷, ÷÷÷ or ÷÷÷÷	÷	—
22	—	—	—	—	22	—	—	22
16	—	÷÷	4	7	5	15	—	1*
8	±	÷÷	—	6	2	8	—	—
4	÷	÷÷	1	2	1	4	—	—
18	÷÷	÷÷	6	9	3	17	1*	—
5	÷÷	÷÷÷	4	1	—	5	—	—
2	÷÷	÷÷÷	2	—	—	2	—	—
75 (total)							

* Treated case.

TABLE II

ANALYSIS OF 521 DISCREPANCIES OBTAINED WITH UNTREATED CASES

	Initial test				Three weeks later			No. of sera not obtained
No. of sera	W.R.	Kahn	M.L.	No. of sera	W.R.	Kahn	M.L.	
260	—	—÷	—	6	—	÷÷	—	51
				203	—	—	—	
24	±	—	—	22	—	—	—	2
155	—	—	÷	64	—	—	—	91
32	—	—	÷÷	3	÷÷	÷÷	÷÷÷	2
				17	—	—	—	
				8	—	—	÷	
				2	±	÷—	÷—	
34	±	—	÷—	2	——	——	÷÷	23
				9	±	÷	——	
16	—	÷	÷—	1	——	÷—	÷—	3
				12	—	—	—	
521 (total)				349				172

M.L.—Modified Laughlen.

RESULTS

The above modification of the Laughlen reaction has been tested with 16,824 sera, in parallel with the Wassermann (Wyler, 1929, 1931) and Kahn tests. Of these, 11,496 were diagnostic specimens and 5,328 from cases undergoing antisyphilitic treatment. In the former group, all three tests gave negative results with 374, while 521 sera produced discordant results of one kind or another. These discrepancies have been analysed (Table II), and are discussed later. In the treated group, 1,460 specimens were positive with the three tests, and 2,448 negative. 1,420 sera gave discrepancies which are analysed in Table III.

Discussion

This modified Laughlen (M.L.) test is intended mainly for use as a quick and convenient screen test. To be useful, its sensitivity level must be such that no serum from a new, untreated case escapes detection, even though it may be classed as doubtful or weakly positive by other tests for syphilis. If the M.L. test is to be used for the control of treatment, its sensitivity must in every instance be at least as high as that of any standard test. Non-specific positives are to be expected in both instances, but, if comparatively few in number, they are of little importance because all positive sera should be re-tested by the Wassermann test, the Kahn test, or both.

Accordingly, the results of the investigation may be discussed in relation to untreated and treated cases.

TABLE III

DISCREPANT RESULTS WITH SERA FROM 1,420 TREATED CASES

	W.R.	Kahn	M.L.
765	—	—	+ +
582	—	+ +	+ +
37	—	+ +	—
6	+	+ +	+
13	±	+ +	—
17	±	—	+ +
1,420 (total)			

M.L.—Modified Laughlen.

Untreated cases.—No serum giving a positive Wassermann reaction was negative by the M.L. test. Direct agreement between the two methods was found in 10,975 tests, plus 260 (Table II), a total of 11,235 or 97.7 per cent. If the 121 cases in which re-testing produced agreement are added to this total the percentage rises to 98.5, and it might be greater if more of the discrepant sera had been available for re-testing. In no instance was there any evidence of an earlier specific response to the Wassermann test than to the M.L. test. the 24 doubtful Wassermann results being accepted as non-specific on the evidence of the later tests. Of the 155 sera giving doubtful M.L. readings, all those available for re-testing were found negative, which confirms the belief that this degree of precipitation can usually be disregarded. Nevertheless it is a useful reaction to record, but does not imply that further examination of a serum is necessary unless this is indicated by suggestive clinical evidence.

A similar comparison with the Kahn test showed that no specific positive or " doubtful " result was ever found in a syphilitic serum giving a negative M.L. test. The large number, 260, of non-specific Kahn results in this series was due probably to the large number of sera from pregnant women. But if the doubtful M.L. results are disregarded substantial agreement was achieved with the Kahn test in 96 per cent of examinations, or in 98 per cent if the " three weeks later " results are taken into account.

Thus in a large series of serum examinations the M.L. test proved 100 per cent sensitive. Specificity in a screen test is of less moment. The number of non-specific positive M.L. results simply indicates the sera which have to be unnecessarily re-tested by Wassermann, Kahn, or both. In this series 82 positive M.L. reactions were associated with negative or doubtful Wassermann-Kahn results. Examination three weeks later showed that at least eight of such results were specific, therefore only 74 sera, approximately 0.7 per cent of the total, would have been re-tested unnecessarily by an accepted standard procedure had the M.L. test been used for screening. In one contingency, however, it may be desirable to know the actual non-specific rate of this test. If an immediate report is required—as, for instance, on a patient leaving the district, or on a blood donor prior to a direct transfusion—it may be helpful to know what reliance can be placed on this quick and convenient test. Its non-specific rate here was approximately 12 per cent. The Kline exclusion test has a non-specific rate of 9 per cent and the Hinton
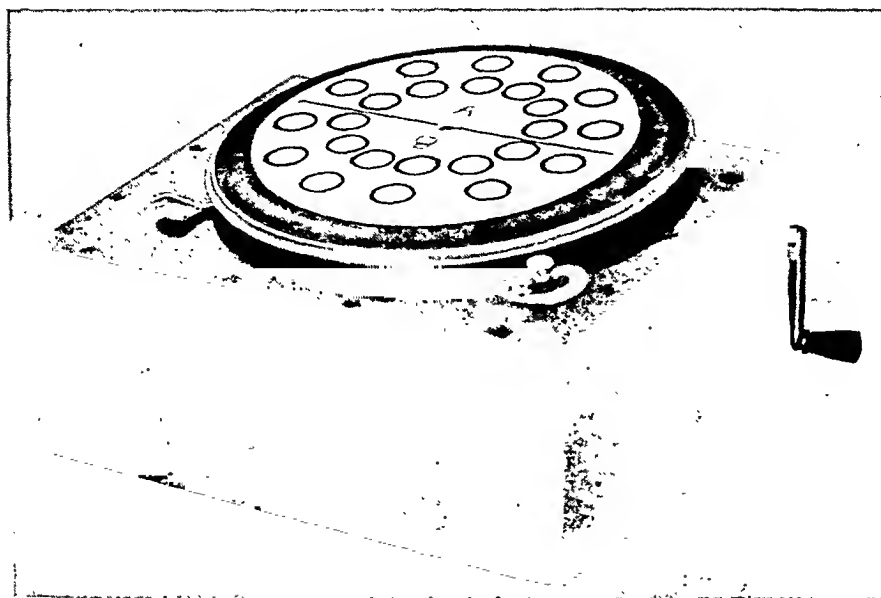
(*a*).—The mechanical mixer.
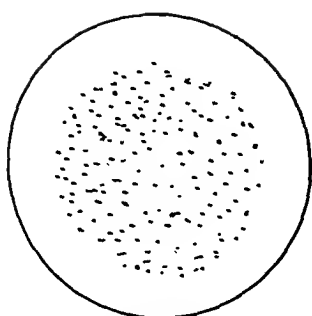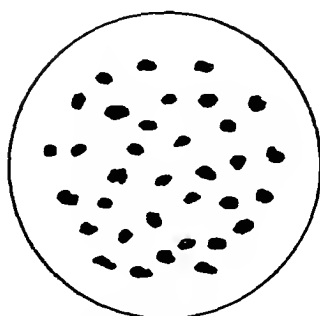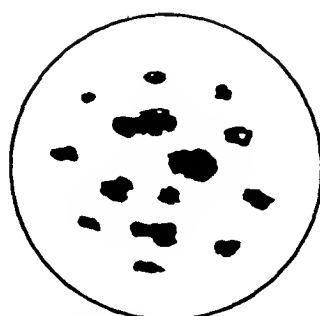
| Positive (+ +). | Positive (+ + +). | Positive (+ + + +). |

(*b*) The various degrees of positive precipitation. All about ×2 magnification.

PLATE III

N

and Kahn presumptive test approximately 5 per cent. But in this series the non-specific rate of the standard Kahn was unusually high at 10 per cent, so that the high rate of the M.L. was probably due more to the nature of the material examined than to any deficiency in the test. Nevertheless this finding supports the belief that complete sensitivity and specificity are not attainable in any one simple test. Should a report be required at short notice it is, however, permissible to use the M.L. test if only complete precipitation, the + + + + reading, be accepted as positive, and all intermediate grades of precipitation classed as doubtful. All positive and negative readings will then be entirely specific.

Treated cases.—Complete agreement with the Wassermann was recorded in approximately 73 per cent of the specimens examined, and this low rate of agreement between the two tests was due to the many instances where the M.L. remained positive after the Wassermann and Kahn had become negative (Table III). On the other hand, 13 sera which were positive in varying degrees with both Wassermann and Kahn, and 37 sera positive with Kahn only, gave negative results with the M.L. Although the Laughlen reaction usually remains positive after the Wassermann and Kahn have become negative, about 1 per cent of treated cases react negatively to the Laughlen while the other tests are still showing a positive result. This irregular sensitivity, and especially the poor correlation with the Kahn test, so widely applied to the control of antisyphilitic treatment, suggests that this version of the Laughlen test is unsuitable for use with treated cases.

Summary

The composition of the Laughlen antigen and the technique of performing the test have been investigated. From the findings a modified procedure has been evolved, which has been tested in the examination of 16,824 sera, 5,328 from treated cases, 11,496 from untreated cases. The findings suggest that the modified Laughlen test has no special merits as a final diagnostic test, that it is not suitable for the control of treated cases, but that it is especially valuable as a " screen " test for diagnostic specimens. The degree of sensitivity is such that no syphilitic serum is missed, yet comparatively few non-specific positives are encountered. The antigen is easy to prepare by a standardized method and gives constant results for at least four months from its date of manufacture. The whole test is extremely simple to perform.

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SINGLE-SAMPLE TESTS IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

BY

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Many attempts have been made to compare the usefulness of the various tests of liver function. The papers of White and others (1940), Wilson (1940), Higgins and others (1944), Maizels (1946), and Sherlock (1946) discuss the merits of such diverse estimations as bromsulphalein, hippuric acid, and urobilinogen excretion ; bilirubin, cholesterol, cholesterol esters, differential proteins, fibrinogen, phosphatase, phospholipins, and prothrombin in serum or plasma ; galactose and fructose tolerance : and the cephalin-cholesterol, colloidal red, Takata-Ara, and thymol turbidity tests. It has been difficult to find an independent measure of the degree of liver damage, and even the liver biopsy used by Sherlock and others may not always be satisfactory. If the tests are regarded as empirical aids in specific diagnostic problems. rather than as a means of measuring liver damage, the situation is simplified, though the evaluation of results must often depend upon the accuracy of clinical diagnosis.

The object of the present work has been to investigate the suggestion of Maclagan (1944a) that a combination of the thymol turbidity or the colloidal gold test with an estimation of the serum alkaline phosphatase would distinguish between infective hepatitis and obstructive jaundice in a high proportion of cases. Maclagan (1947) has published a survey of his results using these tests on two hundred cases of jaundice, of which fifty-six were obstructive and ninety-five infective hepatitis. He was able to distinguish between obstructive and non-obstructive jaundice in from 65 to 79 per cent of cases by combining one flocculation test with an estimation of the serum alkaline phosphatase. The limits set for the data to be considered of diagnostic significance were so severe as to exclude the possibility of misclassification within his group of cases. In practical work it is usual to accept some possibility of error as inevitable, and with this in mind Maclagan's results are very satisfactory. Rennie and Rac

(1947) have reported their experience with the cephalin-cholesterol and colloidal gold tests in obstructive jaundice, infective hepatitis, and several other diseases involving liver injury. They showed that these two flocculation tests gave negative results in 84 per cent of cases of obstructive jaundice while positive flocculations were obtained with nearly as high a proportion of sera from patients with infective hepatitis. These authors point out that in spite of general agreement between the results of the two tests they were not always parallel, and a similar lack of strict correlation has been observed among the flocculation tests used in the present investigation.

In addition to the thymol turbidity and colloidal gold tests the present investigation has included Britton's (1945) modification of the Takata-Ara reaction, together with the estimation of the alkaline phosphatase, bilirubin, and albumin-globulin ratio. These six tests can all be performed on 3.5 ml. serum and have in this respect an advantage over the tolerance tests, which involve several venepunctures. The results have been analysed statistically by methods described by Bradford Hill (1946).

Experimental

METHODS

All colours and turbidities were read in a King (1942) photo-electric colorimeter. using Ilford spectrum light filters.

Takata-Ara reaction.—The modified method of Britton (1945) was used. Three tubes containing respectively 1 ml. of 1:2. 1:4. and 1:8 dilutions of serum in 0.85 per cent NaCl were treated with 0.25 ml. 10 per cent Na₂CO₃ and 0.3 ml. 0.5 per cent HgCl₂. The tubes were shaken after each addition and examined after twenty-four hours. The amount of precipitate in each tube was graded as 3. 2, 1, or 0, corresponding to the more usual notation of $+$, $\pm$, trace, and 0. This device was adopted for statistical reasons. the result of the test being obtained by totalling the grades of the three tubes.

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Colloidal gold test.—The method and grading of results were those of Maclagan (1944b).

Thymol turbidity test.—Maclagan's method (1944a) was used. The buffer made up as described by Maclagan gave a *p*H of less than 7.8, so the *p*H was adjusted to this value with NaOH. It was later found that this led to a reduction in sensitivity, but for purposes of comparison it was considered inadvisable to alter the procedure in the middle of the investigation.

Alkaline phosphatase.—This was estimated by the method of King and others (1942).

Differential serum proteins.—The biuret method of Robinson and Hogden (1940) was used for total proteins and albumin.

Bilirubin.—This was estimated by the method of King and others (1937).

Results

NORMAL BLOOD DONORS

The three flocculation tests have been carried out on the sera of one hundred apparently normal blood donors, and the results are shown in Table 1. (The large number of significant figures given in this and other tables are included for statistical purposes and not because they are supposed to have physical significance.) It has been assumed that the " normal range " is given by the mean ± twice the standard deviation. When a result outside the normal range was obtained with one of the flocculation tests on the serum of a normal blood donor the other two tests were usually normal.

TABLE I

RESULTS OF FLOCCULATION TESTS ON ONE HUNDRED SERA FROM NORMAL BLOOD DONORS

Test	Mean	Standard deviation	Mean ± 2 × S.D.
Takata-Ara	0.4	0.92	0-2
Colloidal gold ..	0.3	0.81	0-2
Thymol turbidity ..	1.2	0.53	0.1-2.3

S.D. = standard deviation.

Flocculation tests and albumin-globulin ratio. —It has been stated by Bálint and Bálint (1942) that positive results in the Takata-Ara reaction are merely due to an abnormally low albumin-globulin ratio, and in particular to a relative excess of " euglobulin," which includes the fraction now referred to as γ-globulin. It was thought useful to compare the results of all three flocculation tests with those of differential serum protein estimations, and correlation coefficients have been calculated between the individual flocculation tests and between these tests and

TABLE II

CORRELATION COEFFICIENTS OF FLOCCULATION TESTS WITH ALBUMIN-GLOBULIN RATIO, AND OF FLOCCULATION TESTS WITH ONE ANOTHER

Correlation	Number of tests (n)	Correlation coefficient (r)	"Significance" $\left(r \Big/ \sqrt{\dfrac{1}{n-1}} \right)$
Gold–A/G	187	0.22	3.0
Thymol–A/G	187	0.23	3.1
Takata–A/G	186	–0.38	5.2
Takata–Thymol	352	+0.49	9.2
Takata–Gold	320	+0.61	10.8
Thymol–Gold	315	+0.77	13.7

the albumin-globulin ratio. Pathological sera were used, and the results are shown in Table II.

The correlation coefficient divided by its standard error (col. 4) is in each case greater than 2 and is therefore significant. This does not prove, however, that positive flocculation tests are due to a common cause, and still less does it prove that the cause is a low albumin-globulin ratio. The lowest correlation coefficients in the table are those between the albumin-globulin ratio and the flocculation tests, and the highest correlation is between the colloidal gold and thymol turbidity tests. A reduced albumin-globulin ratio is more closely associated with a positive Takata-Ara reaction than with high results in the colloidal gold and thymol turbidity tests, and this point will be referred to in the discussion.

INFECTIVE HEPATITIS AND OBSTRUCTIVE JAUNDICE

Flocculation tests and proteins.—A comparison between results from patients with infective hepatitis, diagnosed clinically, with those from cases of obstructive jaundice proved at operation or necropsy, is shown in Table III. If a series of investigations had been done on the same patient the first complete set was used for the statistical analysis. It has been assumed that the difference between two means $(\bar{x} - \bar{y})$ is significant if $\bar{x} - \bar{y}$ divided by the standard error of the difference (S.E.) is greater than 2. While all 3 flocculation tests gave significant differences in the two forms of jaundice the colloidal gold and thymol turbidity tests gave much better discrimination than the Takata-Ara reaction.

The serum proteins and albumin-globulin ratio were almost identical in the two forms of jaundice.

Bilirubin and phosphatase.—The mean serum bilirubin in infective hepatitis (6.8 mg./100 ml.) was significantly lower than that observed in obstructive jaundice (12.2 mg./100 ml.), but the

TABLE III

COMPARISON BETWEEN RESULTS IN INFECTIVE HEPATITIS AND IN OBSTRUCTIVE JAUNDICE

		Takata-Ara (grade)	Colloidal gold (grade)	Thymol turbidity (units)	Total protein (%)	Albumin (%)	Globulin (%)	A/G ratio	Alkaline phosphatase (units/100 ml.)	Bilirubin (mg./100 ml.)
Infective hepatitis	n	37	32	37	28	28	28	28	38	36
	$\bar{x}$	4.49	2.84	4.81	6.44	3.25	3.20	1.14	17.59	6.80
	S.D.	2.22	1.73	2.99	0.93	0.90	0.97	0.47	7.94	5.67
Obstructive jaundice	n	34	31	34	25	25	25	25	33	34
	$\bar{y}$	2.06	0.06	0.78	6.18	3.12	3.05	1.05	37.85	12.19
	S.D.	2.14	0.25	0.49	0.67	0.56	0.55	0.29	16.36	9.45
Analysis of differences	$\bar{x}-\bar{y}$	2.43	2.78	4.03	0.26	0.13	0.16	0.09	20.26	5.39
	S.E.	0.52	0.31	0.50	0.23	0.20	0.21	0.11	3.13	1.88
	$\dfrac{\bar{x}-\bar{y}}{\text{S.E.}}$	4.7	9.0	8.1	1.2	0.7	0.8	0.8	6.5	2.9

n = Number of patients. $\bar{x}$ and $\bar{y}$ = mean values. S.D. = standard deviation. S.E. = standard error of difference between means. A/G = albumin-globulin.

serum alkaline phosphatase was of much greater discriminant value than the bilirubin.

Sherlock (1946) found that no positive correlation existed between the bilirubin and phosphatase contents of the serum in obstructive jaundice and infective hepatitis. This observation has been confirmed by the present work. In obstructive jaundice the correlation coefficient was 0.29, and the coefficient divided by its standard error was only 1.63, indicating that the correlation was not significant. The corresponding figures for infective hepatitis were 0.17 and 1.00. The number of cases on which these figures are based (72), combined with those of Sherlock, makes it virtually certain that bilirubin and phosphatase are not secreted into the serum by the same mechanism, which supports Maclagan's (1947) view that the rise in phosphatase "represents a positive secretory response of the liver cell to a variety of chemical or bacterial insults." An alternative explanation would be a difference in the mechanism of the removal of these substances from the plasma.

Relative discriminant value of tests.—If we regard $(\bar{x}-\bar{y})/$S.E. as a measure of the relative discriminant values of the tests, the descending order of usefulness given by the figures in Table III is: colloidal gold, thymol turbidity, phosphatase, Takata-Ara, and bilirubin. Maclagan (1947) found that the alkaline phosphatase had the highest discriminant value, followed by thymol flocculation, colloidal gold, and thymol turbidity. Unfortunately the thymol flocculation was not recorded frequently enough in the present work for adequate statistical treatment.

MISCELLANEOUS DISEASES

Although many pathological sera have been examined the number for each disease has been small except for infective hepatitis and obstructive jaundice. Malaria and infective mononucleosis, and to a lesser extent amoebic hepatitis and thyrotoxicosis, have given positive flocculation tests, while extensive carcinomatous metastases in the liver and almost total destruction of normal hepatic tissue in Banti's disease have given results within normal limits.

Discussion

Takata-Ara reaction.—In the series of eighty-one cases considered by Bálint and Bálint (1942), positive results in the Takata-Ara reaction were nearly always associated with a lowered albumin-globulin ratio. Only six anomalous results were obtained—four sera with albumin-globulin ratios of 1.3 to 1.6 gave positive results, whereas no flocculation was observed with two sera having albumin-globulin ratios of 1.1. Although the present results show a highly significant correlation (– 0.38) between the Takata-Ara reaction and the albumin-globulin ratio, there were many examples in which the results were not parallel. Among 188 sera, twenty-eight had a Takata-Ara reaction lower than grade 2, coupled with albumin-globulin ratios below 1.2, and on the other hand Takata-Ara reactions higher than Grade 2 occurred with twenty-four sera having albumin-globulin ratios higher than 1.2. Britton's Takata-Ara reaction cannot therefore be regarded merely as a qualitative indication of lowered albumin-globulin ratio.

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Colloidal gold and thymol turbidity tests.—There is disagreement concerning the mechanism of the flocculation tests, particularly regarding the thymol turbidity test. The evidence was reviewed by Maclagan and Bunn (1947), who found that γ-globulins were the main precipitating proteins in the thymol, gold, cephalin-cholesterol, and Takata-Ara tests. The cephalin-cholesterol and Takata-Ara reagents were also precipitated by α- and β-globulins from hepatitis sera. The gold test was inhibited by albumin and α- and β-globulins, while albumin inhibited the cephalin-cholesterol and Takata-Ara reactions. Cohen and Thompson (1947) believe that although the γ-globulins are raised in hepatitis sera while the β-globulins may only be slightly above normal, nevertheless a high thymol turbidity is probably due to a qualitative change in the β-globulin fraction. Unfortunately the conditions used by Cohen and Thompson were not the same as those specified by Maclagan.

The present investigation shows that the colloidal gold and thymol turbidity tests are not measures of exactly the same phenomenon. The correlation coefficient of + 0.77 between these two tests (Table II) is high, but in many individual cases they gave discordant results. Among 323 pathological sera, twenty gave abnormally high thymol turbidities combined with normal " golds," whereas eight had high " golds " with normal " thymols." Thus in about 9 per cent of these sera the results of the two tests were not in agreement, which would be unlikely if both depended on the same set of conditions. The flocculation is probably due in both cases to the same positive cause (e.g., γ-globulins), but the modifying factors such as albumin and α- and β-globulins are different (Maclagan and Bunn, 1947 ; Moore and others, 1945). The danger of drawing sweeping conclusions from comparisons between parallel results of these empirical tests is illustrated by the fact that slight variations in reagents and technique may give very different results with the same serum.

The technical difficulty of fractionating sera electrophoretically is a serious obstacle to quantitative investigation of the flocculation tests. The work of Maclagan and Bunn (1947) was based upon results from a single mixed specimen of " typical " hepatitis serum, and the limitation which this places upon their interpretations will be realized when it is considered that in a series of cases of " typical " infective hepatitis the results of the various flocculation tests may range from all being strongly positive to an occasional example in which all are negative (Tables IV and V).

TABLE IV

ANOMALOUS RESULTS IN INFECTIVE HEPATITIS

No.	Takata (grade)	Gold (grade)	Thymol (units)	A/G	Alkaline phosphatase (units/100 ml.)	Bilirubin (mg./100 ml.)
1	1	1	1.8	2.1	10.0	5.5
2	2	0	0.9	0.9	15.8	4.3
3	0	0	0.4	2.4	33.2	6.1
4	3	0	1.4	1.2	13.6	1.4
5	0	1	1.8	—	17.4	6.0
6	0	—	1.1	—	19.6	3.6
7	3	0	3.2	1.4	16.5	21.0
8	5	0	—	1.5	13.4	—
9	5	3	2.1	1.3	16.4	9.7
10	2	2	2.1	1.6	9.7	4.1
11	3	—	1.6	—	12.5	4.6
12	5	—	2.1	—	24.0	2.5

Differential diagnosis of jaundice.—A comparison is shown in the Figure between the results obtained with the three flocculation tests when applied to sera from normal blood donors and from patients with obstructive jaundice and infective hepatitis. The means are shown at the corners of the triangles, and in the middle of each side is given the " significance " ($\bar{x} - \bar{y}$/S.E.) of the difference between the means at the corresponding corners. The Takata-Ara reaction in *both* forms
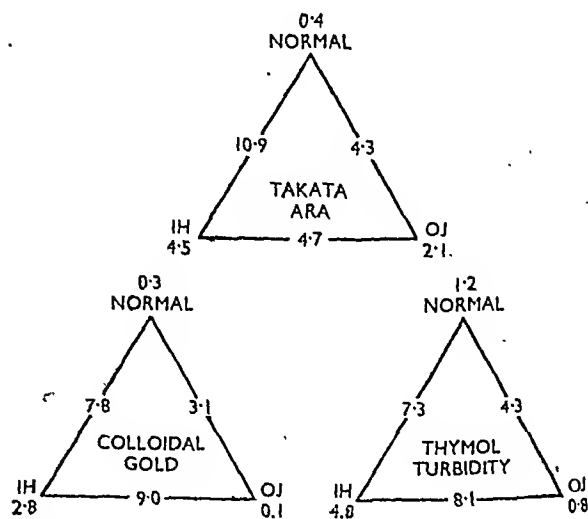
FIGURE.—Differences between results of flocculation tests in normal individuals and in patients with obstructive jaundice and infective hepatitis. (The figures at the corners of the triangles are means, and those on the sides represent significance of differences between means.)

of jaundice was significantly above the normal, which is consistent with the fact that liver damage occurs in both conditions. However, in the case of the thymol turbidity and colloidal gold tests the results in obstructive jaundice were significantly *lower* than the normal values. These two tests should not, therefore, be referred to as "liver function tests."

For the differentiation of obstructive jaundice from infective hepatitis these results suggest that in obstructive jaundice the colloidal gold test will be grade 0, or occasionally grade 1, the thymol turbidity 2.1 units or less, and the alkaline phosphatase greater than 25 units/100 ml. In infective hepatitis the colloidal gold will be greater than Grade 1, the thymol turbidity above 2.1 units, and the phosphatase less than 20 units/100 ml. No case of obstructive jaundice has been encountered which would have been wrongly diagnosed using either the colloidal gold or thymol turbidity test alone with the above criteria, but among thirty-nine cases diagnosed clinically as infective hepatitis no less than twelve gave misleading results with one of these flocculation tests (Table IV). In the first five cases shown in the table both tests gave results within normal limits. However, in ten of these twelve cases the phosphatase was below 20 units/100 ml., whereas among thirty-three cases of proved obstructive jaundice a phosphatase of less than 20 units/100 ml. only occurred five times, the lowest value observed being 16.8 units/100 ml. Taking all the criteria into consideration the only cases shown in Table IV which would be likely to be wrongly diagnosed are numbers 3, 6, and 12. and only in Case 3 are the results really typical of obstructive jaundice. Serial tests were done on seven of the twelve cases in Table IV, and three of these eventually gave positive flocculation tests.

The frequency of positive results for the Takata-Ara reaction in obstructive jaundice makes this test useless for the differentiation of the cases quoted in Table IV. Among thirty-three obstructive jaundice sera, fourteen gave results above Grade 2, and nine above Grade 3. It is noteworthy that the albumin-globulin ratios shown in the table are, with only one exception, within normal limits, which suggests that the liver damage could not have been severe, and in all cases except two the bilirubin is below the mean for infective hepatitis of 6.8 mg./100 ml.

That the chance of mis-classification, using the above criteria, is real and not due to mistaken clinical diagnosis is illustrated in Table V, which shows results from two soldiers, A and B, who entered hospital within a few days of one another. They were close friends and slept in adjoining beds in barracks, and there seems little doubt that they had infective hepatitis of common origin. The results of the tests on the two men were very different even when done simultaneously, and, if the colloidal gold and thymol turbidity results alone had been considered. A might well have been diagnosed as suffering from obstructive jaundice. The low phosphatase made such a diagnosis unlikely, but it must be admitted that it is a mistake to place too much reliance on this test when it conflicts with the flocculation tests. In doing so one would be discarding the results of two tests of high discriminant value in favour of that of a single test of lower discriminant value (Table III).

Maclagan (1947) gave a series of criteria for discrimination which excluded all possibility of mistaken diagnosis within his group of cases. A similar classification of the results obtained in the present work shows that, in round figures, all cases with phosphatase greater than 40 units/100 ml.

TABLE V

TWO CONTRASTED CASES OF INFECTIVE HEPATITIS, PROBABLY OF COMMON ORIGIN

Case	Date	Takata-Ara (grade)	Colloidal gold (grade)	Thymol turbidity (units)	Total protein (%)	Albumin (%)	Globulin (%)	A·G ratio	Alkaline phosphatase (units/100 ml.)	Bilirubin (mg./100 ml.)
A	1.8.46	1	1	1.8	8.4	5.7	2.7	2.1	10.0	11.1
	2.8.46	2	1	1.8	8.7	5.8	2.9	2.0	13.3	13.5
	7.8.46	1	0	1.2	—	—	—	—	—	—
	13.8.46	1	0	1.6	7.5	4.8	2.7	1.8	8.9	2.5
B	6.8.46	6	3	4.7	6.8	4.0	2.8	1.5	20.6	6.6
	7.8.46	6	4	4.8	—	—	—	—	—	15.0
	13.8.46	4	5	5.0	7.5	3.9	3.6	1.1	17.6	39.2
	24.8.46	4	1	2.4	6.7	3.8	2.9	1.7	13.6	4.1
	5.9.46	4	1	2.0	5.9	3.8	2.1	1.8	10.9	2.9

were obstructive jaundice and all with phosphatase less than 17 units/100 ml. were infective hepatitis. All sera having colloidal gold tests less than Grade 2 and thymol turbidities less than 2.2 units, combined with phosphatase higher than 33 units/100 ml., were from patients with obstructive jaundice, and all cases having colloidal gold tests greater than Grade 1 and thymol turbidities greater than 2.1 units were of infective hepatitis. These results agree well with those of Maclagan, except that Maclagan's thymol and gold reagents were evidently considerably more sensitive than those used in this investigation.

Summary

1. The usefulness of the Takata-Ara reaction, colloidal gold, and thymol turbidity tests, differential serum proteins, serum alkaline phosphatase, and serum bilirubin estimations for the differential diagnosis of infective hepatitis and obstructive jaundice has been investigated.

2. Statistical analysis indicates that all these tests except the differential serum proteins give significantly different results in the two forms of jaundice, but only the colloidal gold, thymol turbidity, and alkaline phosphatase are of high discriminant value.

3. The three flocculation tests are closely correlated with one another and, to a lesser extent, with the albumin-globulin ratio. Each is a measure of a different complex of factors, and none is merely a qualitative test for a lowered albumin-globulin ratio.

4. The Takata-Ara reaction gave results above normal in both forms of jaundice, but the mean results of the colloidal gold and thymol turbidity tests were significantly lower in obstructive jaundice than in normal sera.

5. There is no significant correlation between the bilirubin and the phosphatase in either form of jaundice.

6. All sera from patients with obstructive jaundice had colloidal gold tests less than Grade 2 and thymol turbidities less than 2.1 units, and 75 per cent had a serum alkaline phosphatase greater than 25 units/100 ml. About 70 per cent. of sera from patients with infective hepatitis had colloidal gold tests greater than Grade 1, thymol turbidities greater than 2.1 units, and phosphatase less than 20 units/100 ml. All cases with phosphatase greater than 40 units/100 ml. were obstructive jaundice, and all with phosphatase less than 17 units/100 ml. were infective hepatitis. All cases with gold tests less than Grade 2 and thymol turbidities less than 2.2 units, combined with phosphatase higher than 31 units/100 ml., were obstructive jaundice.

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TECHNICAL METHODS

THE ESTIMATION OF POTASSIUM BY A MICRO-METHOD

BY

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A method for the estimation of potassium using a silver cobaltinitrite complex was described previously (Cumings, 1939). The method as described below has overcome the disadvantages of the original method, which were the need for ashing sera and the rather difficult titration involved. The composition of the compound has been ascertained and will be indicated in this paper.

Breh and Gaebler (1930), after deproteinization of serum, added silver nitrate followed by the usual sodium cobaltinitrite reagent, and part of their procedure has been utilized. A colorimetric method is used in place of final titration for estimation of nitrite (Hoagland and Ward, 1942 ; see also Looney and Dyer, 1942).

Method

To 3.1 ml. of distilled water in a centrifuge tube are added 0.2 ml. serum followed by 0.3 ml. 2/3 N-H_2SO_4 and 0.3 ml. 10 per cent w/v $Na_2WO_4.2H_2O$; after mixing, 0.1 ml. of 5 per cent w/v $AgNO_3$ is added, and the contents mixed and centrifuged ; 2 ml. of the supernatant fluid are placed in a centrifuge tube graduated at 10 ml., and 1 ml. of my original silver cobaltinitrite reagent is added (Cumings, 1939), and the tube centrifuged after standing for one hour. To the precipitate are added about 10 ml. of 50 per cent acetone, which is centrifuged after mixing, and this procedure is repeated once more, after which 1 ml. of 2.5 N-NaOH is added to the precipitate and the tube placed in a boiling-water bath for five minutes. Distilled water is now added up to the 10 ml. mark. After mixing, the contents are centrifuged and 1 ml. of the supernatant fluid is diluted to 25 ml. with distilled water.

Five ml. and 10 ml. of this dilution are placed in test tubes made up to 10 ml. with water, and 1 ml. of 0.2 per cent sulphanilamide is added, followed by 0.2 ml. of 0.1 per cent. N-(1-Naphthyl)-ethylenediamine hydrochloride.

A blank of 10 ml. of distilled water, and two

standards of 10 ml. of distilled water containing 0.004 and 0.008 mg. of sodium nitrite, are treated in the same way, and all tubes are placed in ice or in a refrigerator as at this temperature there is a greater constancy of colour production. After cooling 0.2 ml. of 6N-HCl are added to each tube, the contents mixed, and the tubes returned to the cold for fifteen minutes, after which time readings are taken in a colorimeter. (A King pattern photo-electric colorimeter has been found to be very suitable.) The pink to red colour is permanent for twenty-four hours.

A standard potassium solution should also be treated in the same manner as the serum by taking 0.2 ml. of a solution containing 51.7 mg. KNO_3 per 100 ml. (equivalent to 20 mg.K./100 ml.). With a visual colorimeter the calculation $\frac{s}{u} \times 20$ will give the result in mg./100 ml.

Results

Table I gives a few of the results obtained using this method, my original method, and that of King and others (1942).

TABLE I

COMPARISON OF RESULTS OF K IN SERUM BY THE METHOD OF KING AND OTHERS AND OUR METHODS

King	$K_3Ag_2Co_2Na(NO_2)_{12}$ method	
Mg./100 ml.	Original method mg./100 ml.	New method mg./100 ml.
18.0	21.0	19.5
25.3	26.6	26.6
24.1	24.0	25.0
18.0	19.0	20.7
27.0	25.6	26.3
20.4		21.6
20.6	21.3	21.6
19.2	21.6	19.8
19.0	17.0	18.0
40.0	41.0	42.0
(Haemolysed serum)		

N***

Composition of Compound

Samples of the potassium silver cobaltinitrite compound were prepared from known amounts of potassium nitrate, dried at 100° C., and weighed. Separate quantities of the compound so prepared were then analysed for potassium, silver, cobalt, sodium, and nitrite, and Tables II and III show the results obtained. The analytical methods used are indicated in the appendix.

TABLE II

MOLECULAR WEIGHT OF COMPOUND FORMED
(Assuming molecule contains 3 atoms K)

KNO_3 taken	Compound formed	Molecular weight
0.2636 g.	0.8695 g.	1020
0.0188 g.	0.0638 g.	1028

TABLE III

COMPOSITION OF COMPOUND

Constituent	Estimated (%)	Calculated (%)
K	10.94	11.40
Ag	20.1	21.05
Co	12.0	11.50
Na	2.2	2.24
NO_2	52.8	53.80

The results found by experiment and by calculation are in sufficiently close agreement to substantiate the view that the formula $K_3Ag_2Co_2Na(NO_2)_{12}$ with a molecular weight of 1026 is correct.

Discussion

The method presented is quick and reliable, and can be performed on 0.2 ml. quantities of serum.

If much less serum were used the amount of precipitate would be so small that any losses during centrifugation would cause a relatively large error.

The final pink colour is very easy to match and the method involved is an extremely delicate one for the estimation of nitrite.

Summary

A method for the estimation of potassium in small amounts of serum is described.

The potassium silver cobaltinitrite compound formed has been shown to have the formula $K_3Ag_2Co_2Na(NO_2)_{12}$.

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Appendix

ANALYTICAL METHODS USED

Sodium : King, 1946. *Micro-Analysis in Medical Biochemistry*, Churchill, London.

Cobalt : 1. Gravimetrically as $CoSO_4$.
2. Colorimetrically with choline chloride and sodium ferrocyanide (King, 1946. *Micro-Analysis in Medical Biochemistry*, Churchill, London).

Silver : 1. Gravimetrically as AgCl.
2. By titration with thiocyanate.

Nitrite : Application of Bratton-Marshall sulphonamide method, as used by Hoagland to determine nitrite.

Potassium : From titration of silver with thiocyanate. See original paper (Cumings, 1939) for rationale.

THE ENUMERATION OF BLOOD PLATELETS

BY

H. S. BAAR

The platelets of human blood can, with reference to their fragility, be divided into two groups. The first group comprises the·smallest platelets with very high fragility described for the first time by Floessner (1922). In the second group are the medium-sized and large platelets which have a higher degree of resistance and show in normal individuals a typical curve of disintegration (Baar and Szekely, 1929). The existence of the first group was questioned by Hartmann (1932), who regarded the tiny platelets as fragments of normal blood cells. Steinmaurer (1932) was, however, able to demonstrate by a special method this type of platelet in stained films (compare also Juergens, 1934). Apart from these platelets of normal people, there are also pathological giant forms (found especially in thrombocytopenic purpura) and exceptionally highly resistant pathological micro-platelets. The latter form was seen by Baar (1934) for a short period in a case of essential thrombocytopenia.

Corresponding to the two main types of platelets the technical methods for their enumeration may be divided into two groups. Thus the methods of Floessner (1922), Boshammer (1926), Juergens (1934), Steinmaurer (1932), Cumings (1933), and Olef (1935) permit the enumeration of all platelets, and the number found in normal individuals by these methods is 700,000 to 900,000 per c.mm. All these methods, however, are very laborious and unsuitable for routine laboratory work. Moreover, up to date no pathological conditions are known in which only the number of the most fragile platelets is decreased or increased without corresponding changes in the other groups of platelets. The much simpler methods of the second group supply us, therefore, with sufficient information. These are either indirect, in which the number of platelets is calculated from their relation to red blood corpuscles in stained films (Fonio, 1912) or in wet preparations (Cramer and Bannerman, 1929; Damashek, 1932; Leitner, 1935; Ladewig, 1935); or direct, in which the platelets are counted in a counting chamber (Spitz, 1921; Langmeyer, 1918; Kristenson, 1924; Tocantins, 1937; Wintrobe, 1946). The enumeration of platelets in Giemsa-stained films has the advantage that

the morphology of platelets can be ·studied at the same time; it is, however, too time-consuming for routine work. The counting chamber methods have, on the other hand, various drawbacks. In some (Gutstein, 1932; Lenggenhager, 1936; Tocantins, 1937; Wintrobe, 1946) the platelets are counted in the presence of red blood corpuscles, which necessitates a comparatively high dilution: in others, agglutination and disintegration of platelets are not sufficiently suppressed. The latter objection applies also to a method described by the present author (1928) which is a combination of the methods of Kristenson and of Langmeyer. Reliable·results are obtained only if the sample is counted immediately after collection. In addition, the diluting fluid deteriorates rapidly in spite of the presence of mercuric chloride, and loses its haemolytic property. Attempts were therefore made to replace urea by another more stable haemolytic substance, and at the same time to inhibit the disintegration of platelets. Saponin was found suitable for the first purpose. It has high erythrocytolytic action even ·in the smallest concentrations. An alteration of platelets, however, can also be noticed. In high concentrations they become dissolved in a short time. In medium concentrations the red cells are dissolved immediately; at first the platelets remain preserved, but after some minutes they lose their high refraction of light, become shadow-like, and finally disappear. In a concentration of 0.025 g. per 100 ml. the erythrocytes are quickly haemolysed while the platelets remain practically unaffected. A simple substitution of saponin for urea in Kristenson's fluid proved, however, impracticable because of the formation of numerous precipitates in the presence of saponin, mercuric chloride, and brilliant cresyl blue. Some of these precipitates can easily be differentiated from platelets and removed by filtration. Others, however, are spherical, pale blue, highly refractile, and can easily be mistaken for platelets; and it was impossible to remove these precipitates by filtration. This difficulty was overcome by the use of formaldehyde as preservative and fixative. Formaldehyde has a "tanning" action on the cell membranes. It thus prevents the disintegration of platelets, but owing to the

same property it counteracts the erythrocytolytic effect of saponin.

Method

A concentration of saponin and formaldehyde was finally found in which the red blood corpuscles are promptly haemolysed without the platelets being affected.

Saponin (B.D.H.)	...	...	0.25 g.
Sodium citrate	...	...	3.5 g.
Concentrated solution of formal-			
dehyde (40%)	...	...	1.0 ml.
Brilliant cresyl blue	...	...	0.1 g.
Distilled water	to		100.0 ml.

The solution must be filtered once after preparation and remains unchanged for many months. The above formula is valid for the B.D.H. saponin only. For other saponins the proper concentration of saponin and formaldehyde can easily be found empirically, bearing in mind the above-mentioned action of these two substances.

For the enumeration of platelets a white cell pipette is used. The counting fluid is first sucked up to the mark 0.6, followed by blood from a finger-prick up to the mark 1.0 and counting fluid again up to the mark 11. The pipette is vigorously shaken for about 3 minutes immediately after collection and again before the counting chamber is filled. The enumeration can be done either immediately after collection or even several hours later without a change in the result. The platelets are easily recognized in the counting chamber by their light refraction and faint blue colour. No red blood corpuscles should be seen in the chamber. A high-power dry objective is used. When counting, the fine adjustment of the microscope must be continuously moved with wide excursions because the platelets are evenly distributed throughout the whole depth of the chamber (0.1 mm.). In cases with normal numbers of platelets it is sufficient to count half a square millimetre, but with thrombocytopenia the whole chamber or even both sides of a Buerker chamber should be counted. The dilution is 1 in 25, and the number of platelets in one square millimetre has therefore to be multiplied by 1,000 and divided by 4. The white blood cells are well stained and can be enumerated at the same time. Heparinized blood cannot be used.

Although no known method for enumeration of blood platelets can claim to be ideal, the present method has proved to compare favourably with many others which have been tried, so far as quickness and reliability are concerned. It has now been used for many years by junior technicians at the Children's Hospital, Birmingham.

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MODIFICATIONS OF THE WEINBACH METHOD FOR THE DETER-MINATION OF SODIUM IN SERUM

BY

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The Weinbach (1935) method, based on that of Barber and Kolthoff (1928, 1929), involves the precipitation of sodium as the triple salt uranyl zinc sodium acetate in the presence of alcohol, and subsequent titration of this salt with sodium hydroxide, using phenolphthalein as indicator. We have found the end-point of the titration to be unsatisfactory, and have preferred colorimetric methods for this part of the determination. The colorimetric procedure, described by McCance and Shipp (1931), which depends on the colour produced by the addition of potassium ferrocyanide to a solution of the uranyl zinc sodium acetate in the presence of a small quantity of acetic acid, was found reasonably satisfactory so long as we used visual colorimetry, and compared the solution to be determined with a standard solution similarly treated, at the same time. When, later, we began to use photo-electric methods, difficulties arose owing to the rather rapid changes which take place in the intensity of the colour produced by this method. Indeed, the adoption of modern methods of colour measurement which do not involve direct comparison of two solutions made up under similar conditions at the same time has brought to light greater degrees of instability of many coloured solutions than were at first recognized. King and Garner (1947), for instance, have drawn attention to the fading of the blue colour of the solution used in many blood-sugar methods—a phenomenon which was immediately evident to us when we abandoned the usual visual colorimetric method for evaluating these solutions.

The change which occurs in the sodium determination by the ferrocyanide method is indicated in the Table, which gives the readings obtained on the same solution at different time intervals after adding the ferrocyanide ; the instrument used is the Spekker photo-electric absorptiometer.

TABLE

VARIATION OF SPEKKER READINGS WITH TIME, FOLLOW-ING ADDITION OF POTASSIUM FERROCYANIDE TO SOLUTION OF TRIPLE ACETATE IN PRESENCE OF ACETIC ACIO

Time (minutes)	Spekker reading
3	0.359
8	0.367
13	0.377
18	0.388
23	0.406
28	0.427

In view of these changes we decided to examine the method described by Darnell and Walker (1940). In this method the precipitated uranyl zinc sodium acetate, after suitable washing, is dissolved in water and treated with 5 per cent sulphosalicylic acid solution and 10 per cent sodium acetate solution. A clear, bright yellow solution results which we found to be very stable, and the curve given by readings with the Spekker instrument with sodium solutions corresponding to serum sodium concentrations over the range likely to be found in human blood was a straight line. The method has been in use here for some time and has proved to be very satisfactory.

More recently Bradbury (1946) has published a useful simplification of the uranyl zinc acetate procedure. He measured the colour of the supernatant fluid after the precipitation of the triple acetate, and so determined the reduction of colour produced by the precipitation: this reduction of colour was proportional to the amount of sodium present in the solution examined. This modification effects a useful saving of labour compared with earlier procedures, and, moreover, it avoids possible losses, either mechanical or by solution in

washing fluid, whieh are associated with the washing process.

We have found it neeessary to make certain alterations in the proeedure recommended by Bradbury. We have found that, with the quantities of reagent and alcohol reeommended by him, precipitation of the triple salt is by no means complete in the twenty to thirty minutes he specifies. We have increased the proportion of alcohol and generally varied the volumes of fluids used so as to get complete precipitation, and at the same time to have readings of the eolour of the supernatant fluid which shall give the most convenient range with the Spekker instrument when applying the method to serum sodium determinations. Even with the increased proportion of aleohol we find it necessary to let the mixture stand for two hours in the refrigerator at approximately 3° C. to ensure complete precipitation. The readings we have obtained with sodium solutions corresponding to serum coneentrations of 200 to 400 mg. per 100 ml. fall on a straight line.

The full details of the method we use are as follows :

Method

To 1 ml. of serum add 1 ml. of water and 1 ml. of 20 per cent trichloracetic acid solution. Mix and allow to stand for 10 minutes ; filter. To 1 ml. of filtrate in a small conical flask add 2 ml. of the Weinbach uranyl zinc acetate reagent,[1] 3 ml. of absolute aleohol, and 2 ml. of water. Mix thoroughly ; stopper the flask, and allow it to stand in the refrigerator for two hours. Centrifuge the contents of the flask and determine the colour of the supernatant fluid in the Spekker photo-eleetric absorptiometer, using the Ilford violet filter 601. Subtract the Spekker reading of this fluid from the reading obtained with a similar mixture except that 1 ml. of water replaees the 1 ml. of filtrate, and obtain the sodium content of the serum by reading off the difference on the curve obtained by plotting the differences obtained from a series of standard sodium solutions.

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[1] *Preparation of Weinbach's uranyl-zinc-acetate reagent. Solution A :* 77 g. of uranyl acetate and 14 ml. of glacial acetic acid are dissolved with gentle heating and stirring in 400 ml. of water, and volume made up to 500 ml. in a volumetric flask. *Solution B :* 231 g. of zinc acetate and 7 ml. of glacial acetic acid are dissolved by gentle heating and stirring in 400 ml. of water, and volume made up to 500 ml. in a volumetric flask. The two solutions are mixed while hot, allowed to stand twenty-four hours or longer, and filtered.

INTERNATIONAL SOCIETY OF HEMATOLOGY

The International Society of Hematology will hold its biannual meeting at the Hotel Statler, in Buffalo, New York, from August 23 to 26, 1948. The following time has been tentatively allotted for symposia and presentations: half a day on general subjects, including radioactive and stable isotopes in haematology, half a day for problems and diseases related to the red cells, half a day for problems and diseases related to white cells, one day for immuno-haematology, Rh-Hr (CDE-cde) antigens and antibodies and haemolytic anaemias, half a day for coagulation problems and haemorrhagic diseases, and half a day for the business meeting.

Applications for the presentations of scientific exhibits are now being received by Dr. O. P. Jones, Department of Anatomy, University of Buffalo, Buffalo, New York. Chairman of the Programme Committee is Dr. Ernest Witebsky, Buffalo General Hospital, Buffalo, New York.

Dr. Eduardo Uribe Guerolo, Leibnitz 212, Nueva Colonia Anzurez, Mexico, D.F., is in charge of the programme from South and Central America, and Sir Lionel Whitby, University of Cambridge, England, is in charge of arrangements for the programme from Europe. Communications concerning applications for the programme will be received by the above-named committee men.

All scientific sessions and exhibits will be open to scientists interested in haematology. This will, of course, include members of the medical profession and those branches of science dealing with haematology, such as biochemistry, biophysics, genetics, immunology, etc.

Communications from interested haematologists in the United Kingdom, and applications concerning membership, should be sent to Dr. Robert R. Race, Lister Institute, Chelsea Bridge Road, London, S.W.1.

REVIEWS

The Background of Therapeutics. By J. Harold Burn. Geoffrey Cumberlege, Oxford University Press. 1948. Pp. 367. Price 22s. 6d.

This is not merely another book on therapeutics, but an attempt to describe the scientific background to recent therapeutic advances. The author is therefore frequently involved in discussions on bacteriology, as in the excellent review on methods of sterilization, and on chemistry, biochemistry, and pathological processes; all of which are in a clear style which is simple and easy to follow.

The author, in the preface, states that the work was undertaken after impressions, gained in America, of the importance placed in that country on laboratory and experimental work, and their value in improving diagnosis and treatment. He has used mainly British work as his basis, and has provided many British references in the hope that "Americans may learn of some of the work done in this country which ought to be more widely known." His approach to clinical research, which shows a full understanding of laboratory methods, will be found very acceptable to pathologists. It is clearly impossible to cover all the modern views on many varied subjects in 340 pages, but the condensation is on the whole admirably done. There are, however, several chapters which may well be recast in a future edition. The order of chapters might with benefit be arranged, so that disinfection, for example, is not squeezed between thyroid and allergy. The compound chapters are not all suitably chosen; for example a chapter dealing with plasma proteins and their place in cirrhosis of the liver ends up with the treatment of heart failure with digitalis.

There is an excellent half-chapter on iron metabolism, and it is hoped that in a new edition this will become the basis of a discussion on the therapeutics of the anaemias, instead of ending with a short discourse on calcium metabolism. The outstanding omission is, in fact, the absence of a review on blood and blood products in therapeutics, particularly as so much work on these subjects has been done in this country.

Clinical pathologists will be delighted to find, in a book written for clinicians, so many of the recent advances in investigational and experimental pathology which will give their colleagues an appreciation of the newer trends involving laboratory investigations, many of which will in the near future be in everyday demand in the control of modern therapeutic measures.

A. GORDON SIGNY.

Catalogue of Medical Films. Compiled by the Royal Society of Medicine and the Scientific Film Association. Published by ASLIB, 1948. Price 7s. 6d.

Those of us who know anything of the history of this jointly compiled catalogue have impatiently awaited its appearance. Now that it is available many will appreciate it and will realize that a document of this nature has been much needed in the past.

In this booklet are to be found the titles of some eight hundred films of medical interest, and although a good deal of space is devoted to an elaborate cross-indexing of these titles the net result is that films may be located numerically, generically, or alphabetically. In addition to this, details are given of some two hundred of these films: such details, however, are descriptive and not critical. This is perhaps disappointing, but the difficulties in the way of adequate appraisal must be considerable. It seems that we have not yet reached American standards in this respect ; the American College of Surgeons have already laid down some criteria of medical film appraisal. Critical review, however, sometimes discourages the previewing of films by the teacher himself : this in turn leads to misuse and then to discredit.

The majority of those who refer to this work will be amazed at the wide choice of subject material, and it will now seem as easy to arrange a programme of films for an informal meeting as to select a single available film for the illustration of a specific lecture.

R. J. V. PULVERTAFT.

ASSOCIATION OF CLINICAL PATHOLOGISTS

COMPETITION FOR THE DESIGN OF A PATHOLOGICAL DEPARTMENT

The Association invites entries from interested individuals (e.g., pathologists, technicians, architects, and others) for this competition. Entries by two or more competitors jointly, will be permitted. The essay should include a description and plans for a department of pathology to serve a population of 200,000 to 300,000, and providing: clinical pathology for an "area" or "group" hospital and for general practitioners of the area, including domiciliary service : bacteriology for authorities in public health.

The following subjects and aspects of work should be surveyed: clerical and record services : amenities for patients and staff : bacteriology: biochemistry : haematology : morbid histology : morbid anatomy (including coroner's work and comprising post-mortem room and mortuary, not necessarily within the same building as the rest of the department). The competitor should describe and give detailed plans of type of building and layout of accommodation and should consider light, heat, ventilation, other systemic services, and the attributes and type of material used in building and in internal fixtures and fittings.

There will be first, second, and third prizes of respective values £50, £30, and £20. The competition will remain open until May 1, 1949, by which date all entries should have been sent to the Hon. Secretary, Association of Clinical Pathologists, The Royal Infirmary, Worcester. The plan will remain the property of the competitor, but a copy of the plan and the accompanying essay will remain with the Association.

ABSTRACTS

This section of the JOURNAL is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this JOURNAL some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.

BACTERIOLOGY

"Aerosporin": an Antibiotic Produced by *Bacillus aerosporus* Greer. AINSWORTH, G. C., BROWN, A. M., and BROWNLEE, G. (1947). *Nature, Lond.*, 160, 263.

An antibiotic for which the name "aerosporin" is proposed has been isolated from a Gram-positive spore-forming rod identical with that isolated by Greer from Chicago tap water and called *Bacillus aerosporus*. The organism has been isolated both from Surrey and Yorkshire soil, and can be extracted by methods similar to those used for streptomycin. It shows selective action against Gram-negative organisms, particularly *Bacterium coli*, *Haemophilus pertussis*, and *Salmonella typhi*, as well as against *Brucella bronchiseptica*. It is stated to be bactericidal in action, and weight for weight to have the same order of activity against Gram-negative as penicillin against Gram-positive organisms. Resistant strains could not be obtained by culture methods.　　*R. Wien.*

Polymyxin: A New Chemotherapeutic Agent. STANSLY, P. G., SHEPHERD, R. G., and WHITE H. J. (1947). *Bull. Johns Hopk. Hosp.*, 81, 43.

This antibiotic, specific for Gram-negative bacteria, is produced in shallow or deep culture by the soil organism *Bacillus polymyxa*.

Streptomycin Therapy in Urinary Tract Infections. PULASKI, E. J., and AMSPACHER, W. H. (1947). *Surg. Gynec. Obstet.*, 85, 107.

Of 465 patients with urinary-tract infections treated by streptomycin, 45% were not improved; calculi, pathological affections of the kidney (especially hydronephrosis, and paraplegia accounted for many of the failures. *Bacterium coli* and *B. aerogenes* infections responded well and *Pseudomonas* badly.

Guy Blackburn.

A Streptomycin Sensitivity Test. MATHEWS, A. G., and SHELTON, M. L. (1947). *Med. J. Aust.*, 2, 387.

The authors investigate conditions, such as the suitability of the medium, effect of *p*H, and size of inoculum, affecting the estimation of the sensitiveness of bacteria to streptomycin, and describe a method giving reproducible results.

A Method for Determining Sensitivity to Penicillin and Streptomycin. HOYT, R. E., and LEVINE, M. G. (1947). *Science*, 106, 171.

The authors have devised a method of sensitivity determination which obviates the use of a sterile technique and employs compressed tablets of penicillin and of streptomycin weighing 60 mg. each. The penicillin tablets contain approximately 1, 0.5, and 0.1 Oxford unit, and the streptomycin tablets contain 1, 0.1, and 0.01 mg. respectively. The agar plate is heavily streaked with the organism being tested, and the tablets are dropped into position with forceps. Such material as sputum, exudates, and infected body fluids can be tested directly without preliminary subculture. The plates are incubated, and are read when growth of the organisms is sufficiently advanced to show sensitivity or resistance.

R. Wien.

The Sensitivity of Organisms of the Genus *Leptospira* to Penicillin and Streptomycin. WYLIE, J. A. H., and VINCENT, E. (1947). *J. Path. Bact.*, 59, 247.

Tests were made *in vitro* of the sensitivity to penicillin and streptomycin of 6 strains of *Leptospira icterohaemorrhagiae* and single strains of 23 other species of leptospira of European and Oriental origins. Streptomycin is stable but penicillin deteriorates on incubation, so the potency of the penicillin was estimated at the end of the experiment and it was found to have diminished by one-quarter to one-third of its original strength. Growth was markedly diminished in all cases and a number of strains were completely inhibited. In most cases penicillin was effective in a lower concentration than streptomycin.

The Relation of Protein Binding to the Pharmacology and Antibacterial Activity of Penicillins X, G, Dihydro F, and K. TOMPSETT, R., SCHULTZ, S., and McDERMOTT, W. (1947). *J. Bact.*, 53, 581.

Among these four penicillins the degrees of reduction in antibacterial activity caused by serum and albumin were roughly in direct proportion to the degrees of binding to these substances as demonstrated by dialysis. "The most satisfactory rationalization of these data is that the individual penicillins bind *in vitro* to a varying degree to the albumin content of serum, and that the resulting penicillin-albumin complex has little or no antibacterial activity."

The authors consider other possible explanations of their findings; examine evidence to suggest that binding of penicillin to protein occurs also *in viro*; and say that their finding that penicillin sensitivity is different in different concentrations of serum may have an important bearing on the usual methods of assay of penicillin in serum.

A Study of the Absorption and Excretion of Oral Penicillin in Children. MARKOWITZ, M., and KUTTNER, A. C. (1947). *J. Pediat.*, 31, 195.

It has been shown that gastric acidity is probably not a major factor in the destruction of orally administered penicillin and that the presence of food in the stomach influences penicillin absorption. The authors report their study on the absorption and excretion of oral penicillin as part of an investigation to determine its value as a measure for the prevention of streptococcal upper respiratory infections in rheumatic subjects, and conclude that oral administration of penicillin is a satisfactory procedure in children provided the drug is given under fasting conditions. *A. G. Watkins.*

The Effectiveness of Metachloridine in Suppressing Natural Infections with *Plasmodium malariae* and *P. falciparum* in British Guiana. KENNEY, K., and BRACKETT, S. (1947). *Amer. J. trop. Med.*, 27, 493.

The suppressive effect of metachloridine on infections with *P. malariae* and *P. falciparum* was tested in school-children of a malarious district of British Guiana; infections with *P. vivax* were too scarce to be included. *P. malariae* was found in only one blood film in the treated group, while 35 controls showed the presence of parasites. Fifty days after the end of treatment, however, there were 10 infections in the treated group and 23 in the controls. The effect on *P. falciparum* was much less, and it was necessary to double the dose. *P. falciparum* was found in 20 blood films of the first group and in 33 of the controls, and in 17 treated with the double dose compared with 60 controls. No toxic effects of metachloridine were seen in any of the trials.

Streptobacillus moniliformis Bacteremia with Minor Clinical Manifestations. LEVINE, B., and CIVIN, W. H. (1947). *Arch. intern. Med.*, 80, 53.

A case of rat-bite fever caused by *Streptobacillus moniliformis* is described. The clinical phenomena include an incubation period of a week, fever, fleeting arthralgia, a rash, and very little local inflammation. Penicillin is curative. *G. F. Walker.*

Cultivation of Tubercle Bacilli from Gastric Juice. A Study of the Factors affecting the Cultivation of *Mycobacterium tuberculosis* from Gastric Juice. VINCENT, V., and BIRGE, E. A. (1947). *Amer. Rev. Tuberc.*, 55, 556.

The authors show that the survival of the tubercle bacillus depends on the acidity of the gastric juice: the more acid the specimen the shorter the survival time. To eliminate false negative reports gastric specimens should be examined immediately. When this is not possible, neutralization of the sample by the addition of sterile N/10 NaOH until the pH is 6.5 to 7.0 will allow the tubercle bacillus to live for several days. *R. J. Lumsden.*

Aetiology and Pathogenesis of Vincent's Angina and of Ulcero-membranous Stomatitis. (Zur Aetiologie und Pathogenese der Vincent'schen Angina und der Stomatitis ulceromembranosa.) MELCZER, N. (1947). *Dermatologica*, 94, 13.

The author demonstrated elementary bodies in smears from 26 cases of Vincent's angina. Animal inoculation with material from the lesions was negative, but 70% of the human cases reacted to intradermal injections. Bacteria-free filtrate of exudate mixed with bacterial suspensions caused phagedena-like ulcers. It is concluded that virus plus bacteria are responsible in symbiosis in the lesions.

Pathogenic Mechanism of Infective Phlebitis and the Genesis of Septicaemia. (Le mécanisme pathogénique des phlébites infectieuses et la genèse des septicémies.) REILLY, J., and GRISLAIN, J.-R. (1947). *Ann. Med.*, 48, 113.

The authors, struck by the failure to produce phlebitis experimentally by the intravenous injection of organisms, decided to use the approach from the adventitia. They conclude that bacterial toxins and various soluble products of tissue breakdown can reach a vein from without, alter the endothelium, and so lead to thrombus formation. Any organisms circulating in the blood are entrapped in this thrombus. Thus an intravenous dose of organisms which in the healthy animal would be without apparent effect leads to infection of the thrombus and proliferation therein. The subsequent changes depend on the proteolytic activity of the organism used; if this is slight, further thrombosis occludes the infected portion, and after a brief bacteriacmia the blood remains sterile; if the organisms cause disintegration of the thrombus, then the progression is to pyaemia and fatal septicaemia. *A. C. Lendrum.*

Factors Influencing the Red Cell Agglutination-inhibitive Reaction in Influenza and their Application to the Diagnostic Test. WHITMAN, L. (1947). *J. Immunol.*, 56, 167.

Though a considerable number of modifications have been made to the original red-cell agglutination test for influenza, the author considers that a need exists for a standard procedure simple enough to permit its widespread employment. He describes such a procedure. The PR 8 strain of influenza A virus and the Lee strain of influenza B virus were used in all experiments. *F. O. MacCallum.*

Experience with Vaccination Against Influenza in the Spring of 1947. A Preliminary Report. FRANCIS, T., SALK, J. E., and QUILLIGAN, J. J. (1947). *Amer. J. publ. Hlth.*, 37, 1013.

Anticipating an outbreak of type-A influenza in the winter of 1946-7, the authors vaccinated 10,328 students with a vaccine having a red-cell agglutinating titre of 5120. 7615 students acted as controls. Excellent antibody responses were obtained, but investigations of the febrile illnesses showed no significant differences in the incidence of respiratory disease between the vaccinated and unvaccinated groups.

Early Immunization against Pertussis. ADAMS, J. M., KIMBALL, A. C., and ADAMS, F. H. (1947). *Amer. J. Dis. Child.*, 74, 10.

The authors conclude that pertussis immunization in the first six months of life is a safe, practical, and desirable procedure. Reactions to inoculation were negligible. The weekly inoculations gave rapid agglutination response. It remains to be seen whether the titre level can be maintained by giving antigen at monthly intervals during the early months of infancy.

Aetiology of Typhoid Osteitis in Children Confirmed by Bone-marrow Culture. (Etiologia de las osteitis tifieas en el nine comprobada por el mielo-cultivo.) JAUREGUY, M. A., and SIMON, G. (1947). *Arch. Pediat. Uruguay*, 18, 298.

The authors found bone-marrow culture for diagnosis of typhoid fever so useful that they performed a Widal test, blood culture, and marrow culture as a routine. Out of 162 cases examined, 9 remained with persistently positive bone-marrow cultures.

Studies in the Pathogenesis of Rheumatic Fever. The Antistreptolysin Titre in Acute Tonsillitis and Rheumatic Fever. (In English.) WINBLAD, S., MALMROS, H., and WILANDER, O. (1947). *Acta med. scand.*, 196, 533.

The investigation of antistreptolysin titre of 71 cases of acute tonsillitis was made serially using Ipsen's modification of Kalbak's method. Readings of over 200 were considered to be raised, and 52 cases showed some such elevation. Of the 14 who developed rheumatic complications, 12 had an initial titre of 140, and 9 a titre of 200 or more, suggesting previous infections with haemolytic streptococci.

Smear and Culture Diagnosis in Gonorrhoea. McLEOD, J. W. (1947). *Brit. J. vener. Dis.*, 23, 53.

In a series of 275 cases of suspected gonorrhoea in which 50% of the combined smear and culture results were positive, 42% of the cultures were positive against 29% of the smears; the cultures from the cervix and urethra were both positive in 22%, whereas only 7% of smears were positive from both these sites; from the urethra alone, the cultures were positive in 32% against 24% of positive smears; and the cervical cultures alone were positive in 33% against 16% of positive cervical smears. These figures show that diagnosis by culture is superior to that by smears and that the percentage of error is lessened when both methods are used together.

The author draws attention to Stuart's method (*Glasgow med. J.*, 1946, 27, 131) of keeping gonococci under conditions of reduction and so preventing the oxidation which, with drying, is lethal to them.

T. Anwyl-Davies.

The Laboratory Diagnosis of Leprosy. McDONALD, S. (1947). *Trans. R. Soc. trop. Med.*, 40, 451.

Laboratory methods of confirming a clinical diagnosis of leprosy comprise: (*a*) the examination of skin smears stained by the Ziehl-Neelsen method, which is especially suitable for lepromatous and major tuberculoid forms; (*b*) the lepromin test; (*c*) Wassermann and Kahn tests; (*d*) skin biopsy.

BIOCHEMISTRY

Insulin Resistance. AXELROD, A. R., LOBE, S., ORTEN, J. M., and MYERS, G. B. (1947). *Ann. intern. Med.*, 27, 555.

Insulin resistance has occurred in conditions favouring the destruction of insulin in the body, particularly associated with abnormal leucocyte activity. Investigating 3 cases of spontaneous insulin resistance, the authors found no evidence of an insulin antagonist in the sera of their patients. Whilst the aetiology remains unknown no specific therapeutic measures are available. Single large doses are recommended in treatment, rather than repeated smaller doses, and glycosuria and acidosis may require as much as 1,000 to 5,000 units of insulin.

Serum Potassium, Magnesium, and Calcium Levels in Diabetic Acidosis. MARTIN, H. E., and WERTMAN, M. (1947). *J. clin. Invest.*, 26, 217.

The changes in the levels of serum potassium, magnesium, and calcium were studied in 14 patients, aged 12 to 69 years, undergoing treatment for severe diabetic acidosis. The blood-sugar levels varied between 285 and 1,029 mg. per 100 ml. and the insulin requirement in the first 24 hours between 110 and 1,000 units; large amounts of fluid (4 to 12 litres) were given parenterally in the first 24 hours. There was a marked fall in the serum potassium in 46% of patients; critically low values (1.9 and 2.18 milliequivalents) were found in some. The minimum was usually reached after 12 to 24 hours. No constant correlation between total calcium concentration and ionized calcium was found. A marked fall in the serum-magnesium concentration was seen in 36% of the patients during treatment; concentration returned to normal very slowly. It is suggested that potassium and magnesium salts should be given as adjuvants to therapy, but this is contraindicated in patients in shock and with decreased renal function, because dangerously high blood levels may be produced. *A. Schott.*

Carbohydrate Metabolism in the Undernourished. (Der Kohlehydratstoffwechsel bei Unterernährten.) GÜLZOW, M. (1947). *Z. ges. inn. Med.*, 2, 91.

The first part of this paper is a study of 45 cases of inanition, mostly with oedema, 25 of which were fatal. Hypoglycaemia was marked and sugar-tolerance tests always gave a poor response with a flat curve and a very low and delayed peak. After about 1 month on an ample diet in hospital injections of adrenaline and insulin gave normal responses.

A further study was made in order to ascertain the site of the metabolic lesion in starvation, and the observed failure of the tissues to assimilate sugar is attributed to damage to cell enzymic processes and to diminution in hormonal action, especially insulin insufficiency.

The Effect of Ingested Mineral Oil on Plasma and Vitamin A. ALEXANDER, B., LORENZEN, E., HOFFMANN, R., and GARFINKEL, A. (1947). *Proc. Soc. exp. Biol., N.Y.*, 65, 275.

Continued ingestion of mineral oil as such or in the form of mayonnaise dressing by 20 subjects on a normal unrestricted diet resulted in a moderate decrease in plasma carotene concentration. Vitamin A levels remained unchanged. It is concluded that simultaneous ingestion

of mineral oil with food prevents substantial amounts of food carotene from entering the body.—[Authors' summary.]

A Study of Thiamine in Paediatrics. (Studie o thiaminu v pediatrii.) HOUŠTĚK, J. (1947). *Čas. Lék. čes.*, 86, 1006.

Present methods of vitamin B_1 estimation are reviewed. Although physico-chemical methods with thiochrome are most often used, the author prefers Schopfer's micro-biological technique because of its specificity and accuracy. It is based on the fact that *Phycomyces blakesleeanus* requires thiamine for growth but is unable to synthesize it.

Breast-milk seems the only source of thiamine for the infant. Synthesis of thiamine in the intestine is doubtful. Breast-milk of 233 mothers of all classes had a vitamin B_1 content of 15 to 19 μg. per 100 ml. Some differences were encountered in various age groups. Colostrum is free from vitamin B_1. The vitamin content increases from 13.8 μg. per 100 ml. in the first week of lactation to 20.2 μg. per 100 ml. after the second month. Saturation of the mother with 50 to 100 mg. thiamine by oral, intravenous, or intramuscular administration raises the level in breast-milk transiently. It is highest after intravenous injection. The blood level remains lower than the level in the milk.

The vitamin-B_1 content of the cerebrospinal fluid was estimated in 105 children of all ages with various affections of the nervous system. The values were fairly constant and ranged from 1 to 4 μg. per 100 ml. independent of age, condition, or cell and protein content of the liquor. The diffusion of thiamine into the spinal fluid was demonstrated. After intravenous doses of 50 mg. vitamin B_1 the level increased from 1 to 2.5 μg. to 12.5 to 16.5 μg. per 100 ml. With lower doses the increase was negligible.

The thiamine level in blood (usually capillary) was examined in 180 children. A level of 5 to 8 μg. per 100 ml. was found. Values below 4 μg. per 100 ml. are considered to reveal a latent hypovitaminosis. Premature infants had a relatively high level (mean value 9.8 μg. per 100 ml.). The estimation of thiamine in blood is considered unsatisfactory and inconclusive, even with Schopfer's method. The vitamin-B_1 equivalent is recorded as a whole, without distinction of free and fixed vitamin and of intermediate metabolites.

M. Dynski-Klein.

A Study of the Fasting-hour Excretion of Thiamine in the Urine of Normal Subjects. PAPAGEORGE, E., and LEWIS, G. T. (1947). *J. Nutrit.*, 34, 301.

The authors have estimated the 24-hour urinary excretion of thiamine by 63 normal university students, and have compared the values with the amount in a "fasting-hour" specimen, collected in the hour following the 24-hour period and after an overnight fast of about 12 hours. There is a good correlation between the values. In 20 of their 63 subjects the authors also determined the percentage of a 1-mg. oral dose of thiamine excreted in the next 4 hours in the fasting state. The authors therefore confirm previous work which indicates that the fasting-hour excretion is a convenient method in nutritional surveys of obtaining an approximate indication of the trend of thiamine levels in the body. They suggest that the critical level of fasting-hour excretion of thiamine is 4 μg. *H. M. Sinclair.*

Factors Influencing the Urinary Excretion of Calcium. I. In Normal Persons. KNAPP, E. L. (1947). *J. clin. Invest.*, 26, 182.

The normal urinary excretion of calcium was studied under standard dietary conditions in 606 normal persons ranging in age from 1 to 80 years. The mean urinary calcium excretion increased with both age and intake; in adults the increase in excretion with increase in intake is greater than in children. With constant daily calcium intake the mean urinary excretion increased with age throughout the period of growth. The skeletal weight was found to be the factor responsible for these differences as well as for those with sex. The urinary calcium values calculated as a percentage of calcium intake were plotted against the calcium intake per kilo of body weight; it was found that $\dfrac{\text{urinary Ca} \times 100}{\text{Ca intake}}$ was an exponential function of, and varied inversely with, the intake per kilo. The position of urinary calcium values in any individual person relative to the mean depended on the endocrine balance of the person and was independent of age or calcium intake. Normal infants, irrespective of a low or high calcium intake per kilo, had a level of urinary excretion within the range of normal for older subjects but below the mean for this latter group, the mean level characteristic for older subjects being reached at about the age of 2. Undernourished children had a uniformly low calcium excretion, which rose sharply as the child gained weight. Ingested acids (ammonium chloride, ketogenic diet) and a high calcium/phosphorus ratio, without altering the total calcium content of the diet, increased urinary calcium. Some other dietary factors of less importance in urinary calcium excretion are discussed.

A. Schott.

Metabolism of Women during the Reproductive Cycle. XIII. The Utilization of Niacin during Lactation. CORYELL, M., RODERUCK, C., HARRIS, M. E., MILLER, S., RUTLEDGE, M. M., WILLIAMS, H. H., and MACY, I. G. (1947). *J. Nutrit.*, 34, 219.

Niacin intake, secretion in milk, and excretion in urine were studied in 7 healthy nursing mothers during the first 10 days post partum, and in 9 women during periods of 5 consecutive days 2 to 10 months post partum.

The average daily intakes of niacin and its precursor during the 5-day periods ranged from 13 to 23.4 mg. The results show during the first 10 days post partum rapid increases in the amounts of niacin secreted daily in milk, averaging from 0.04 mg. on the first day post partum to 2.9 mg. on the tenth; these figures portray the increases both in concentration and in volume of milk secreted during the puerperium. The average daily niacin content of mature milk ranged from 0.52 to 2.02 mg.; these values, also, show a general relationship to milk volume rather than to intake, and illustrate the wide range of normal variation in the composition of human milk from different mothers and from the same mother at different times. *J. Parness.*

Oral Smears Compared with Vaginal Smears. Case Report. RUSSELL, P. B. (1947). *Sth. med. J.*, 40, 561.

This is a case report to illustrate the value of Russell and Bennett's method in controlling the synthetic oestrogen therapy of menopausal symptoms in a woman of 44, following surgical operation. Gram-stained smears from the surface of the mouth show the influence

of hormonal stimulation on the buccal epithelium, and permanent preparations can be made for comparison. It is suggested that the method is applicable to the oestrogen therapy of vulvo-vaginitis in children, and that the patients themselves could prepare the smears.

E. T. Ruston.

The Effect of Trauma and Disease on the Urinary 17-Ketosteroid Excretion in Man. FORBES, A. P., DONALDSON, E. C., REIFENSTEIN, E. C., and ALBRIGHT, F. (1947). *J. clin. Endocrinol.*, 7, 264.

Analysis of 4,000 determinations of urinary 17-ketosteroid excretion showed that the output was low in chronically ill or seriously undernourished patients. Members of the laboratory staff whose basal output was well established showed a 70% reduction in output during acute infections. The effect of various types of "alarm," including operation, acute infections, etc., showed an immediate rise in excretion in men, followed by a period of subnormal excretion. Women showed no preliminary rise.

The Excretion of 17-Ketosteroids and Gonadotrophin in Children : Normal and Abnormal Cases. HAIN, A. M. (1947). *Arch. Dis. Childh.*, 22, 152.

Gonadotrophin can be detected in normal girls before the seventh year and in normal boys before the ninth. It is excreted in abnormally large amounts in cases of precocious sexual development. Constitutional precocious menstruation is probably due to premature pituitary secretion in association with abnormal ovarian response. Instances of the use of hormone excretion assays in various types of endocrine and constitutional abnormalities are given.

P. R. Evans.

Correlations of Biochemical and Histologic Changes in the Adrenal Cortex. ROGERS, W. F., and WILLIAMS, R. H. (1947). *Arch. Path.*, 44, 126.

The adrenal cortex was studied in a number of healthy persons who died instantaneously after traumatic injury and in whom the glands could justifiably be regarded as normal. Comparisons were made between the cholesterol content and the amount of lipid present, as demonstrated in histological section by use of polarized light, ultraviolet light, and phenylhydrazine and Sudan IV stains in frozen sections. Phloxine-methylene-blue was used in paraffin sections to study vacuolation. The amount of lipid demonstrated by the first three methods was directly proportional to the cholesterol content. Similar correlation was found in glands depleted of lipid in severe toxic states such as fulminating streptococcal septicaemia and by administration of adrenotrophic pituitary hormone.

W. S. Killpack.

Proteins in the Colloidal Gold Reaction. BERNSOHN, J., and BORMAN, E. K. (1947). *J. clin. Invest.*, 26, 1026.

The authors suggest that abnormal colloidal gold reactions are due to a significant change in the γ-globulin content or to an alteration in the proportions of β- and possibly α-globulin to the γ-globulin. A spinal fluid giving the general paretic curve (type I) contains significantly less protective globulins and generally more γ-globulin. A spinal fluid giving the curve found in bacterial meningitides (type III) contains more globulin of the β type than a normal fluid. Other curves of type II can be explained on an alteration of the ratio of the protective to the γ-globulins. The reactions obtained with blood serum in cases of liver dysfunction arise from the relative decrease of protective globulins which are elaborated by the liver cells. The authors suggest that γ-globulin could be used to determine whether or not two batches of gold sol are comparable.

E. T. Ruston.

A Microbiological Method of Standardizing Liver Extracts. MAHDIHASSAN, S., and BAKSHI, V. M. (1947). *Nature, Lond.*, 160, 404.

A bacterium, *Bacterium carotene*, producing a red pigment was isolated from the insect *Cicadella viridis* and grown on artificial media containing liver extract. Growth occurred only when liver extract was added to the medium, and it appeared to be related quantitatively to the concentration of liver extract. The growth factor present in the liver extracts was found to resist autoclaving for 6 hours at 15-lb. pressure, and to be adsorbed on to fuller's earth and activated charcoal but not on to kaolin. It is suggested that this organism may provide a method of microbiological assay for standardizing liver extracts.

Hepatic Dysfunction in Peptic Ulcer. Observations with the Hippuric-acid Test. POLLAK, H. (1947). *Lancet*, 2, 131.

Oral hippuric-acid excretion tests were performed on 68 male patients with peptic ulcer. No other tests of liver function are recorded and no details of the clinical condition of these patients are given, but the author concludes that they had a tendency to depressed liver function, the impairment being greater in the active phase of the disease.

C. Hardwick.

Liver Function Tests in the Diagnosis of Jaundice. A Review of 200 Cases. MACLAGAN, N. F. (1947). *Brit. med. J.*, 2, 197.

Serum alkaline phosphatase shows a maximum deviation from normal in obstructive jaundice, whereas the flocculation tests show maximum abnormality in hepatitis. The present paper is concerned with the results obtained in 200 jaundiced patients, in whom the cause of the jaundice was satisfactorily established by other means. Of these, 56 were cases of obstructive jaundice (37 due to neoplasm) and 118 of acute hepatitis (95 infective hepatitis), and there were also 15 cases of chronic hepatitis and 11 of haemolytic jaundice.

All cases with phosphatase levels above 42 units were obstructive. All with phosphatase levels below 15 units were non-obstructive, and this group included all cases of haemolytic jaundice. All cases with strongly positive flocculation reactions were non-obstructive; all those with weak or negative flocculation reactions and phosphatase levels above 35 units were obstructive.

Douglas H. Collins.

Chronic Liver Disease Following Infectious Hepatitis. I. Abnormal Convalescence from Initial Attack. KUNKEL, H. G., LABBY, D. H., and HOAGLAND, C. L. (1947). *Ann. intern. Med.*, 27, 202.

Reviewing 350 cases of the disease, with a special study of tests of liver efficiency, the authors describe as necessary for the estimation of persistent impairment of the liver a combined use of determinations of the plasma bilirubin level, the bromsulphalein retention test, and the thymol turbidity reaction of the serum. Under an elaborate scheme of chemical testing the authors found

that 17% of their patients sustained relapses or other forms of delayed healing due to functional hepatic impairment; in several, recovery was delayed for over a year. *G. F. Walker.*

A Study of Hepatic Function in Acquired Malaria. SCHNEIDER, L. A., and SHALLENBERGER, P. L. (1947). *Amer. J. med. Sci.*, 214, 80.

Approximately one-third of all acute acquired malaria cases under atebrin therapy are shown to have demonstrable hepatic dysfunction as evidenced by standard liver function test.

Changes in Blood Phosphate after Ingestion of Glucose and Fructose in Sprue. FOURMAN, L. P. R. (1947). *Brit. med. J.*, 2, 411.

Inorganic phosphate concentrations in the plasma, cells, and whole blood after the ingestion of 50 g. of glucose and the ingestion of 50 g. of fructose were estimated in 5 normal subjects and in 4 patients suffering from tropical sprue. Changes in blood "ester" phosphate concentrations and in the rate of phosphate excretion in the urine were also observed.

In normal subjects the plasma phosphate diminished after the ingestion of both glucose and fructose, whereas in the sprue patients after glucose the drop in phosphate was less than in the normal, and after fructose the phosphate remained unchanged or rose. These findings are taken as evidence that in the sprue patients there was a failure of absorption of glucose and fructose. *H. K. Goadby.*

Measurement of the Concentration of Miracil in Biological Fluids. LATNER, A. L., COXON, R. V., and KING, E. J. (1947). *Trans. R. Soc. trop. Med. Hyg.*, 41, 133.

"Miracil D" (l-diethylaminoethylamino-4-methylthioxanthone) has been shown by German workers to be active against schistosome infections in laboratory animals. In view of the possible use of the drug in medicine methods have been worked out for its determination in blood and urine. Blood concentrations may be determined by colorimetric measurement of the amount of bromothymol blue which will combine with the miracil extracted from blood with ethylene dichloride, or by determination of the yellow colour of the drug extracted from the sample. *L. G. Goodwin.*

The Value of Spinal Fluid Examination as a Diagnostic Procedure in Weil's Disease. CARGILL, W. H., and BEESON, P. B. (1947). *Ann. intern. Med.*, 27, 396.

Of 14 cases of Weil's disease observed during a 4-year period, 13 were found to have abnormal spinal fluid. In only 6 were there clinical signs which could be attributed to meningeal irritation. The commonest abnormality was an increase in the cell count. Xanthochromia was noted in approximately 90% of the cases in which jaundice was present. Spinal fluid examination is of value as a routine diagnostic procedure when the diagnosis of Weil's disease is suspected.

On Changes in the Cerebrospinal Fluid during Measles. (In English.) OJALA, A. (1947). *Ann. Med. intern. fenn.*, 36, 321.

After investigating 123 unselected cases of measles without clinical evidence of central nervous system involvement, and 4 cases in which lumbar puncture was called for on clinical grounds, the author concludes that pleocytosis in measles (nearly 30% in his series) is more frequent than has been previously recorded. Changes in the cerebrospinal fluid may be due to the virus itself or its toxins, and the view is expressed that in measles pleocytosis is an allergic phenomenon caused by toxin. The author believes that measles encephalitis is more common than is assumed but that the symptoms are chiefly psychic. *E. H. R. Harries.*

Caronamide for Increasing Penicillin Plasma Concentrations in Man. CROSSON, J. W., BOGER, W. P., SHAW, C. C., and MILLER, A. K. (1947). *J. Amer. med. Ass.*, 134, 1528.

Normally, about 80% of the penicillin in the urine is excreted by the renal tubules and only about 20% by glomerular filtration. The excretion of the drug by the tubules can be completely suppressed by the intravenous administration of either iodopyrin ("diodrast") or p-aminohippuric acid, but the usefulness of this procedure is limited by the large amounts of either of these compounds which have to be injected.

This report describes the clinical application of caronamide (4'-carboxy-phenylmethanesulphonanilide), an orally effective compound of low toxicity capable of producing a reversible inhibition of penicillin excretion by the renal tubules. The effectiveness of caronamide depends upon a "substrate competition between penicillin, which is excreted by the tubules, and 4'-carboxyphenylmethanesulphonanilide, which is essentially refractory to excretion by that transport mechanism."

Of 6 patients studied, 5 showed increases in plasma penicillin concentrations during the period of administration of caronamide. The average concentrations during the period of administration of the new drug were from 2.4 to 7.1 times greater than during the two control periods. *A. L. Walpole.*

Oliguria after Abortion. HUMPHREY, J. H., and JONES, F. A. (1947). *Clin. Sci.*, 6, 173.

Renal failure with oliguria after abortion (of which only 19 cases have been hitherto reported) may be due to pyelonephritis, ingested poisons, sulphonamides, quinine sensitivity, incompatible transfusion, a syndrome with renal changes resembling those of crush syndrome, or symmetrical cortical necrosis. Four patients, all of whom recovered with "conservative" treatment, are fully described. The pathogenesis of this syndrome is discussed in the light of the work of Barclay and others (*Lancet*, 1946, 2, 237). The pathological process involved was thought to be renal vessel spasm with or without thrombosis, such as is found in persons dying with symmetrical renal cortical necrosis.

Studies in Urolithiasis : I. The Composition of Urinary Calculi. PRIEN, E. L., and FRONDEL, C. (1947). *J. Urol.*, 57, 949.

Approximately 700 urinary calculi were studied under the polarizing microscope. Only 9 distinct crystalline substances were found, and these fell into the following groups: oxalates, phosphates, uric acid, urates, and cystine. Detailed descriptions and chemical analyses are given of the stone formations. As many as 4 crystalline compounds were often found by optical analysis in the same calculus.

HAEMATOLOGY

Does Rh-Isoimmunization Cause Early Abortion ? OVERSTREET, E. W., TRAUT, H. F., HUNT, M., and LUCIA, S. P. (1947). *Amer. J. Obstet. Gynec.*, **54**, 235.

The authors conclude that there is no evidence that Rh-iso-immunization may cause abortion in the first 20 weeks of pregnancy.

The Role of a Presumed Serum Protein in the Pathogenesis of Erythroblastosis Fetalis. GUREVITCH, J., POLISHUK, Z., and HERMONI, D. (1947). *Amer. J. clin. Path.*, **17**, 465.

The authors conclude that sera from umbilical cords and from infants less than 6 months of age do not enhance the agglutinating action of immune antibodies as do normal adult sera.

Use of Trypsin in the Detection of Incomplete Anti-Rh Antibodies. MORTON, J. A., and PICKLES, M. M. (1947). *Nature, Lond.*, **159**, 779.

Rh-positive cells sensitized *in vitro* with Anti-D incomplete antibody, and cells from infants suffering from haemolytic disease of the newborn were agglutinated by solutions of trypsin.

Kernicterus. A Follow-up Study of Thirty-five Erythoblastotic Infants. STILLER, R. (1947). *Amer. J. Dis. Child.*, **73**, 651.

Out of 35 children born with erythroblastosis, 29 survived; 4 showed signs of C.N.S. involvement, 2 of these in the neonatal period. Transfusion is of no value in ameliorating damage to the brain.

Modification of Human Red Cells by Virus Action. CHU, C. M., and COOMBS, R. R. A. (1947). *Lancet*, **1**, 484.

After treatment with the haemagglutinating viruses of influenza A and B, swine influenza, and Newcastle disease (N.D.V.), Rh-positive cells were agglutinated by incomplete Rh antibody. The order of activity in this respect was as follows: N.D.V.>swine influenza>influenza B>influenza A. Rh-negative cells were not agglutinated.

The Improved Demonstration of Circulating Antibodies in Hemolytic Anemia by the Use of a Bovine Albumin Medium. NEBER, J., and DAMESHEK, W. (1947). *Blood*, **2**, 371.

Using 20% albumin as diluting fluid, the authors claim to have demonstrated "warm agglutinins" in 5 patients with acquired haemolytic anaemia; in 4 of these no agglutinin was demonstrable when saline was used instead of the albumin. Employing the albumin technique they demonstrated a serum haemolysin in one patient with acquired haemolytic anaemia and in a case of familiar spherocytic anaemia during a haemolytic crisis.

The Significance of the van den Bergh Reaction. GRAY, C. H. (1947). *Quart. J. Med.*, **16**, 135.

The author claims that the terms "prompt," "biphasic," and "delayed" reactions are obsolete, as they depend on the amount of bilirubin present and not on the type of jaundice. The direct-indirect quotient (D.I.Q.), i.e.,

$$\frac{\text{final amount of azobilirubin in the direct reaction}}{\text{final amount of azobilirubin in the indirect reaction}} \times 100$$

is a useful calculation. Below 40, this is diagnostic of haemolytic jaundice; above 50, it indicates obstructive or hepatogenous jaundice. The van den Bergh reaction is quite incapable of differentiating obstructive from hepatogenous jaundice.

Biochemical and Physiological Studies of Two Active Substances Produced by the Spleen. (Études biochimiques et physiologiques sur deux substances actives produites par la rate.) UNGAR, G. (1947). *J. Physiol., Paris*, **39**, 219.

Two physiologically active substances, splenin A and B (*Endocrinology*, 1945, **37**, 329), have been isolated from ox, horse, and sheep spleen and have been produced by an *in vitro* method. Splenin A decreases and splenin B increases bleeding time. Thus, in guinea-pigs one unit of splenin A per kilo injected subcutaneously lowered the mean bleeding time from 131 to 100 seconds, and one unit of splenin B raised it from 131 to 160 seconds.

Splenin A is normally found in the spleen (about 10,000 units per g.), from which it flows into the blood stream and is excreted in the urine. Splenin B occurs in the spleen (about 30 units per g.) and bone marrow (about 30,000 units per g.), but is not normally found in the blood stream. Splenin B is probably identical with thrombocytopen (Troland and Lee, *J. Amer. med. Ass.*, 1938, **111**, 221). Splenin A and B have opposing pharmacological actions on bleeding time, capillary permeability, and haemolysis, the former promoting and the latter retarding combination between the protease and anti-protease of blood. When excess splenin B is produced, it passes into the blood stream and interferes with the mechanism of certain pathological states such as purpura and haemolytic jaundice. Splenin A, the liberation of which may be controlled by the hypophysis and suprarenal cortex, intervenes during the adaptation syndrome probably by neutralizing protease and thus protecting the capillary endothelium. Its pharmacological properties are similar to those of vitamin P substances such as hesperidin and rustin. *J. E. Page.*

Studies on the Enigma of the Hemostatic Dysfunction of Haemophilia. QUICK, A. J. (1947). *Amer. J. med. Sci.*, **214**, 272.

The author describes experiments which in his opinion show that the defect of coagulation in haemophilia is due to lack of thromboplastinogen, the inactive precursor of thromboplastin. Other components are present in normal proportions.

Some Clinical Observations on the Prothrombin Test. FISHER, B. (1947). *Amer. J. clin. Path.*, **17**, 471.

Whole plasma is recommended for use in Quick's one-stage method for the estimation of prothrombin. Dilution with saline, rather than with prothrombin-free plasma, introduces errors. Prothrombin levels should

$$\left(= \frac{302}{\text{prothrombin time in sec.} - 8.7} \right)$$

also be expressed as percentages of prothrombin (Quick), and not in seconds or as percentages of prothrombin times determined in normal subjects.

Significance of the Accelerated Reaction in Determination of Prothrombin Time of Diluted Plasma. TUFT, H. S., and ROSENFIELD, R. E. (1947). *Amer. J. clin. Path.*, **17**, 704.

A 10% dilution of plasma employed in 500 deter-

minations did not indicate that a decreased prothrombin time was correlated with clinical evidence of thrombosis or embolism.

Variations in Prothrombin and Antithrombin in Patients with Thrombosing Tendencies. HURN, M., BARKER, N. W., and MANN, F. D. (1947). *Amer. J. clin. Path.*, 17, 709.

The plasma prothrombin by the one- or two-stage methods and serum antithrombin levels were estimated in 63 patients with thrombotic or embolic conditions. There was no diagnostic correlation between clinical state and laboratory observation.

Variations in Prothrombin and Antithrombin Following the Administration of Dicoumarol. HURN, M., BARKER, N. W., and MANN, F. D. (1947). *Amer. J. clin. Path.*, 17, 712.

The authors compare the one- and two-stage techniques for estimating prothrombin times. The one-stage technique, which depends upon the rate of conversion of prothrombin, is held to be preferable in the control of dicoumarol therapy.

Congenital Hypoprothrombinaemia and Pseudo-hypoprothrombinaemia. QUICK, A. J. (1947). *Lancet*, 2, 379.

The author summarizes his views on blood coagulation, and describes a labile factor (probably identical with Owren's factor 5) whose disappearance causes an increase in prothrombin time when plasma is stored. Two families are described; one suffering from true familial hypoprothrombinaemia; the other from "pseudo-hypoprothrombinaemia." In the latter the prothrombin time was elevated, but prothrombin and the labile factor were present in normal preparations. The lack of still another factor is postulated.

Essential Thrombocytopenic Purpura. Autopsy Findings in Thirty-six Cases. HERTZOG, A. J. (1947). *J. Lab. clin. Med.*, 32, 618.

The post-mortem findings in 36 cases of essential thrombocytopenic purpura are described; these are taken from the records of over 51,600 necropsies performed at the University of Minnesota. Haemorrhages were the chief necropsy findings; in 12 cases intracranial haemorrhages caused death. In 2 cases bleeding from the gastro-intestinal tract predominated. In 1 the principal bleeding was from the renal pelvis and bladder. Nine patients had continued to suffer from widespread haemorrhages after splenectomy. There were no characteristic histological findings. The bone marrow was examined in 10 of the 36 cases. The most constant feature was a marked increase in the number of megakaryocytes. The author suggests that, where the marrow shows a marked decrease in number of megakaryocytes, little or no benefit is derived from splenectomy.

R. Winston Evans.

Influence of Penicillin on the Coagulation of Blood, with Especial Reference to Certain Dental Operations. FLEMING, A., and FISH, E. W. (1947). *Brit. med. J.*, 2, 242.

With high concentrations of penicillin (1,000 units per ml. or more) there is some interference with the clotting mechanism. This effect is of no practical importance in the systemic administration of penicillin. It should be borne in mind when employing solutions of high concentration locally.

Leucocytosis and Changes in the Differential White Cell Count in Renal Colic. (Sul comportamento della leucocitosi e della formula leucocitaria nella colica renale.) BELUFFI, E. L., and FANTONI, S. (1947). *Ann. ital. chir.*, 24, 152.

Cases are cited of right-sided pain due to uncomplicated non-infected ureteric calculi which are associated with leucocytosis up to 14,000 per c.mm. Doubt is thus thrown on the value of the leucocyte count in differentiating appendicitis from ureteric colic.

Idiopathic Thrombocytopenic Purpura. A Study of Three Cases with Special Reference to Changes in the Megakaryocytes. VALENTINE, E. H. (1947). *Amer. J. med. Sci.*, 214, 260.

A decreased formation of platelets by an increased number of megakaryocytes is held to be due to an inhibiting factor secreted by the spleen.

Iron-staining Erythrocytic Inclusions with Especial Reference to Acquired Haemolytic Anaemia. McFADZEAN, A. J. S., and DAVIS, L. J. (1947). *Glasg. med. J.*, 28, 237.

The authors confirm and extend the observations of Pappenheimer *et al.* (*Quart. J. Med.*, 1945, n.s., 14, 75), who described the appearance of numerous stippled red cells in patients with acquired haemolytic anaemia after splenectomy. The basophilic inclusions are found in both nucleated and adult red cells. They react with acid-potassium ferrocyanide and are believed to correspond with the siderotic granules of Gruneberg. Their formation is attributed to some abnormality in haemoglobin formation.

Development of Inclusion Bodies containing Ribose Nucleic Acid in Myeloma Cells after Injections of Stilbamidine. Determination of Stilbamidine in Myeloma Tissue. SNAPPER, I., MIRSKY, A. E., SCHNEID, B., and ROSENTHAL, M. (1947). *Blood*, 2, 311.

Basophilic granules containing ribose nucleic acid not normally present in the cytoplasm of myeloma cells appear after injections of stilbamidine. Methods are given for identifying ribose nucleic acid, and for the estimation of stilbamidine in myeloma tissue.

The Diffraction Method of Measuring Red Blood Cells. PIJPER, A. (1947). *J. Lab. clin. Med.*, 32, 857.

The author reviews the development of the diffraction technique of measuring red-cell diameters and describes an improved apparatus whereby it is possible to calculate the degree and quality of anisocytosis, as well as mean cell diameter.

The Osmotic Resistance (Fragility) of Human Red Cells. PARPART, A. K., LORENZ, P. B., PARPART, E. R., GREGG, J. R., and CHASE, A. M. (1947). *J. clin. Invest.*, 26, 636.

Temperature, pH, and time as well as salt concentration determine the apparent fragility of red blood cells. This paper should be read by all interested in this determination.

MORBID ANATOMY AND HISTOLOGY

Adnexal Carcinoma of the Skin. CHANDLER, N. (1947). *Amer. J. Path.*, 23, 1.

This paper gives an account of the group of "basal-cell carcinomata," or, as the author rightly prefers to call them, "adnexal carcinomata of the skin." The present position as regards nomenclature, classification, and histogenesis of these growths is confused, and the author bases his attempt to bring order out of the existing chaos on a careful study of over 200 tumours. The interesting and fully documented historical review reveals little that is essentially new.

The subdivisions given are as follows : (1) *Pilar Type ;* (2) *Sudoriparous Glandular Type ;* (3) *Basal-celled Type.*

The author's theory, based on his observations and on a consideration of the development of epidermal structures, is that adnexal carcinomata are derived not from basal cells of the epidermis but from the hair follicles, sweat glands, or rather from cells of the embryonal rudiments of these, as opposed to Mallory's hypothesis that they arise from the adult hair matrix.

Mummified Epidermal Cysts (So-called "Calcified Epitheliomas"). KING, L. S. (1947). *Amer. J. Path.*, 23, 29.

A review of the literature relating to "calcified epitheliomata" suggested to the author that several types of these tumours occur. With this in mind he analysed 9 such cases taken from 7,500 consecutive surgical specimens. At the same time 123 epidermal cysts drawn from the same series were also reviewed. The author considers that the term "calcified epithelioma" is misleading and should be abandoned. He suggests that one group of cases be described as "mummified epidermal cysts" and the other group, of which he describes only one, as "basal carcinoma with mummification."

The "Eosinophilic Granulomas" of the Skin. WEIDMAN, F. D. (1947). *Arch. Derm. Syph.*, Chicago., 55, 155.

The diversity of the disease processes in which cutaneous lesions have the histological features of eosinophilic granuloma is emphasized. The term "eosinophilic granuloma of the skin," not to be confused with eosinophilic granuloma of bone, was used originally for an unclassified cutaneous granuloma in which a dense infiltrate, consisting predominantly of eosinophilic cells, was found; histiocytes, monocytes, plasma cells, and mast cells were also present. It is correct to speak of the condition in the plural, and of a given case as "one of the eosinophilic granulomas of the skin." Five hitherto unpublished American cases are described and most of the published cases are quoted. It is suggested that they fall into two broad categories—idiopathic and symptomatic. All the idiopathic cases are thought to be cutaneous manifestations of one or other of the reticuloses; in all of them some eosinophilia of the blood was present. In the symptomatic cases are included cases of Loeffler's syndrome, erythema elevatum diutinum, orificial tuberculosis, non-specific ulceration, yeast infection, and other unclassified inflammatory conditions. Histologically, a disease known as "syphiloid" of cats can be classified as an eosinophilic granuloma.

Whatever underlying disease may be concerned, it is suggested that eosinophilogenic factors may be responsible for concealing or modifying the typical features of the condition. *G. B. Dowling.*

Eosinophilic Granuloma and its Relation to Xanthomatosis Ossea. (Contribución al estudio del denominado "granuloma eosinofílico" y a sus relaciones con la xantomatosis osea.) SCHAJOWICZ, F., and POLAK, M. (1947). *Rev. Asoc. méd. argent.*, 61, 218.

The authors describe the relations between three main types of benign reticulo-endothelial hyperplasia of bone: the solitary granuloma, the eosinophil granuloma, and the local or diffuse types of xanthomatosis ossea which may be accompanied by the Schüller-Christian syndrome or which may rarely complicate Letterer-Siwe's disease.

Using classical staining techniques as well as the silver impregnation methods of Rio Hortega, on material obtained from biopsies and excised tumours, the authors studied the relations of the three types of lesions in 8 cases of their own. Radiologically, the lesions were similar in the different cases, there being destruction of bone with at least one focus in the cranial vault. Histologically, the main finding was the hyperplasia of the histiocytic cells, with infiltration by lymphocytes, plasma cells, and polymorphonuclear leucocytes. Eosinophil infiltration was found in lesions from cases differing widely clinically—for example, in a case by simple extirpation, another with the typically chronic lesions of the Schüller-Christian syndrome, and a third with the acute lesions of Letterer-Siwe's disease. In other cases eosinophils were entirely absent from the lesions. In 2 cases of eosinophil granuloma the authors observed follicles of reticulo-endothelial cells, with transitions to multinucleate giant cells, well demonstrated in the lesions by impregnation with ammoniacal silver carbonate. These appearances were reminiscent of those in sarcoidosis and suggested an inflammatory reaction. The intense eosinophil infiltration might represent an allergic type of reaction to a toxin, tuberculous or other. Xanthomatous transformation appeared mostly in the older lesions and was probably not a permanent change, sometimes disappearing again, either spontaneously or after radiotherapy. Because the xanthoma cells were not always found, even in cases with the Schüller-Christian syndrome, the xanthomatous degeneration was thought to be only a stage in the evolution of the histiocytic granuloma, which, possibly with a stage of giant-cell formation, finally goes on to fibrosis. The xanthomatous change would thus be due to a local disturbance of lipid metabolism, secondary to the inflammatory reaction which the authors believe to be the basis of the histiocytic granuloma. *L. P. R. Fourman.*

Tumours of Sweat Glands. (Tumeurs sudoripares.) DUPONT, A. (1947). *Arch. belg. Derm. Syph.*, 3, 275.

The author divides his cases into three categories: (1) sudoriferous tumours whose structure recalls the excretory portion of the sweat apparatus; (2) sudoriferous tumours of the secreting portion; and (3) tumours which, possessing certain characteristics relating them to sweat glands, evolve towards Malpighian epitheliomata.

The histological details of 11 cases are described and illustrated by drawings.

Ewing's Sarcoma of Bone. LICHTENSTEIN, L., and JAFFE, A. L. (1947). *Amer. J. Path.*, 23, 43.

A study of 17 cases of Ewing's sarcoma of bone is described. Most of the patients were in the second decade of life, 65% being males.

Morphology is described on the basis of four necropsies. Widespread involvement of many bones was often

found in spite of lack of clinical and radiological evidence. Histologically, in all cases crowded cells of uniform size with ill-defined borders were found. They contained little cytoplasm, and their nuclei were large, round, or oval in shape and showed powdery chromatin. Appearances were often variable owing to necrosis and haemorrhage with the subsequent processes of repair.

This description is at variance with Ewing's original description but agrees with that of Oberling.

Metastases were found in the lungs, liver, heart, spleen, kidneys, pancreas, thyroid, and of course in many bones, though it is possible that multifocal origin is the basis of widespread involvement of the skeleton. Lymph nodes were strikingly free from metastases. The breasts, testes, bronchi, gastro-intestinal tract, suprarenals, and sympathetic chain were scrutinized with a view to eliminating the possibility of incorrect diagnosis.

R. B. T. Baldwin.

Endothelial-cell Sarcoma of Liver following Thorotrast Injections. MacMahon, H. E., Murphy, A. S., and Bates, M. I. (1947). *Amer. J. Path.*, 23, 585.

Twelve years after the administration of "thorotrast" for radiographic visualization of the liver a woman aged 70 died suddenly from intra-abdominal haemorrhage from a haemorrhagic lesion of the liver, which the authors regard as sarcomatous. Radioactive deposits of thorotrast were present in the liver, spleen, lymph nodes, bone marrow, adrenals, kidney, and blood-vessel walls. While the diagnosis of sarcoma might be questioned, the case undoubtedly showed widespread damage due to irradiation and haemorrhagic lesions attributable to the thorotrast. *R. A. Willis.*

Multiple Idiopathic Hemorrhagic Sarcoma of Kaposi. Histopathologic Study. Sachs, W., Azulay, R. D., and Convit, J. (1947). *J. invest. Derm.*, 8, 317.

Haemorrhages, angioblasts ("endothelioid cells" by some authors), and spindle cells are described as constant features of Kaposi's idiopathic haemorrhagic sarcoma. The condition may resemble a simple inflammatory process or an angioma, granuloma, or neoplasm, and the suggestion is made that glomus tumour, pyogenic granuloma, and Kaposi's sarcoma may have a common origin; they may be angioblastomata. Kaposi's sarcoma may be considered as a systemic angiosarcomatosis belonging to this group. *G. A. Hodgson.*

Carcinoma in the Wall of a Lung Cavity. (Das Kavernenkarzinom. Seine Bedeutung für den Arzt und für die Begutachtung). Gräff, S. (1947). *Dtsch. med. Wschr.*, 72, 465.

Six cases of cancer in the wall of a lung cavity in males are reported. These carcinomata are apparently often overlooked even at necropsy. *In vivo* the diagnosis is unlikely to be made. All known cases occurred in tuberculous cavities, originating mostly from the point where the bronchus passes out of the cavity. Microscopically a soft or keratinizing squamous-celled carcinoma was found in all cases. This kind of cancer is seen in the small group of cases in which an external stimulant, here starting from the chronically ulcerated wall of the cavity, is the inciting cause of malignancy.

O. Neubauer.

Bronchogenic Carcinoma : Diagnosis by Microscopic Examination of Sputum and Bronchial Secretions ; Preliminary Report. Woolner, L. B., and McDonald, J. R. (1947). *Proc. Mayo Clin.*, 22, 369.

The subject of the identification of tumour cells in sputum and bronchial secretions is reviewed, and the writers record their observations in 70 cases in which there were positive findings. They give illustrative examples of these, and conclude that a positive result may be expected in at least 80% of cases of bronchial carcinoma. [In the opinion of the abstracter this is likely to prove an over-optimistic estimate.] The paper points out that a negative result does not exclude the presence of carcinoma, that considerable experience is necessary to distinguish neoplastic cells from others, and that peripheral carcinomata not involving large bronchi usually give negative results. The authors' findings were negative also in cases of bronchial adenoma. *R. A. Willis.*

Invasion of the Internal Mammary Lymph Glands in Carcinoma of the Breast. Handley, R. S., and Thackray, A. C. (1947). *Brit. J. Cancer*, 1, 15.

In 5 unselected cases of carcinoma of the breast a lymph node of the internal mammary chain was removed through the secondary intercostal space at, or shortly after, radical mastectomy. In 4 cases the internal mammary node was histologically proved to contain carcinoma. In only 2 cases were there axillary deposits. The case in which the internal mammary node was free from growth also had no deposit in the axilla. It is suggested that the technique of radical mastectomy might be modified to include removal of the second-space internal mammary lymph node. Microscopical examination of this node might greatly increase the accuracy of prognosis and prove of assistance in post-operative treatment by irradiation. *R. A. Willis.*

Dysgerminoma Ovarii. Mazel, M. S. (1947). *Amer. J. Obstet. Gynec.*, 53, 1036.

A review of some of the literature on dysgerminoma ovarii is given, with particular stress on the malignancy of the condition and the need for radical operation even in young women. The author reports a case in a single woman, aged 23.

The Question of Theca-cell Tumours of the Ovary and their Hormonal Function. (Zur Frage der Thekazell-tumoren des Ovariums und ihrer hormonalen Funktion.) Limburg, H. (1947). *Z. Geburtsh. Gynäk.*, 128, 186.

Five cases of theca-cell tumours accompanied by post-menopausal hyperplasia of the endometrium are described. In 1 case, 3 years after partial removal of a theca-cell tumour the residue had developed into a simple fibroma. It is probable that all fibromata of the ovary are end-products of theca-cell tumours, analogous to the corpus fibrosum. Oestrogenic ovarian tumours fall into three related groups: (1) pure granulosa-cell tumours, (2) theca-cell tumours, (3) mixed granulosa-cell and theca-cell tumours. Luteinization may take place in any of these. The paper includes an account of a mixed granulosa-cell and theca-cell tumour in a child of 2 years with precocious puberty. *R. Willis.*

Uterine Adenomyosis. Incidence, Symptoms, and Pathology in 1,856 Hysterectomies. HUNTER, W. C., SMITH, L. L., and REINER, W. C. (1947). *Amer. J. Obstet. Gynec.*, 53, 663.

Adenomyosis was found in 517 cases (27.8%) of 1,856 hysterectomies performed for all causes. The two youngest subjects were each 27 and the oldest (carcinoma also present) 73. While most patients were between 41 and 50, 38 were under 35. Since adenomyosis occurs so often in association with leiomyoma of the uterus it is difficult to evaluate its symptomatology. For this purpose 110 cases of advanced adenomyosis without associated pathology were chosen. Of these, 85 had menorrhagia, 61 dysmenorrhoea, 42 metrorrhagia, 23 pain before periods, 9 dysuria and frequency, 7 pain radiating down legs, 6 bearing-down sensations, and 5 nausea and vomiting. Nearly all had either some menstrual irregularity or dysmenorrhoea. A globoid, slightly enlarged, and tender uterus was usually discovered bimanually. A uterus with leiomyoma and adenomyosis was more tender than one with leiomyoma only.

Granuloma of the Fallopian Tube due to Surgical Glove Talc. Silicious Granuloma. ROBERTS, G. B. S. (1947). *Brit. J. Surg.*, 34, 417.

An account is given of granulomatous lesions (2 in abdominal scars and 5 in Fallopian tubes) in which the author ascribes the aetiology to talc from surgical gloves. In spite of a latent period of several years (2 to 17) in which the agent appeared to be dormant, the lesion when activated developed within 2 months in 1 case. The silicious nature of the substance deposited, demonstrated in each case with the polarizing microscope, was proved microchemically in only 1 case. Cultures or animal inoculations were not performed, so that tuberculosis may have co-existed. Amongst other clinical features, pelvic pain and infertility are mentioned.

[The author questions a diagnosis of tuberculosis made on histological grounds only. But if these granulomata are due to talc, the danger of the continued use of this form of glove powder must again be considered.]

Histopathological Study of Prostatic Tissue following Endoscopic Prostatic Resection. BARNES, R. W., BERGMAN, R. T., and FARLEY, S. (1947). *J. Urol.*, 57, 755.

The authors have studied the pieces of tissue removed by transurethral resection of the prostate. They have found that portions removed posteriorly from near the capsule are much more likely to show carcinoma than those from any other site.

Giant Follicular Lymphoblastoma (Brill-Symmers' Disease). PANTRIDGE, J. F. (1947). *Ulster med. J.*, 16, 46.

This is an account of 7 cases of giant follicular lymphoblastoma. The author considers that the disease is little less than half as common as lymphosarcoma, and that some 27% of lymphosarcomata arise from a preceding follicular lymphoblastoma. It is thought that many cases of lymphoblastoma are unrecognized because early sarcomatous change completely obscures the hyperplastic process.

R. B. Lucas.

The Importance of Malignant Degeneration as a Complication of Chronic Ulcerative Colitis. CATTELL, R. B., and BOEHME, E. J. (1947). *Gastroenterology*, 8, 695.

Data are presented suggesting that malignant disease may be found to complicate chronic ulcerative colitis in a small but significant percentage of cases · if clinical observation is sufficiently prolonged.

Pernicious Anemia and Susceptibility to Gastric Neoplasms. KAPLAN, H. S., and RIGLER, L. G. (1947). *J. Lab. clin. Med.*, 32, 644.

The authors review the literature on the coexistence of epithelial gastric tumours and pernicious anaemia, and claim that the two diseases develop in the same individuals more often than would be expected on the basis of chance alone.

The precise nature of the relation between pernicious anaemia and gastric carcinoma is not clear. The authors believe that the evidence suggests that the two diseases are probably linked together by some common factor. Possible factors would include hereditary tendencies, achlorhydria, gastritis, and liver therapy.

R. Winston Evans.

Rare Tumours of the Lacrimal Caruncle. (Seltene Geschwulste der Caruncula lacrimalis.) RADNOT, M. (1947). *Ophthalmologica, Basel*, 113, 270.

Two tumours of the lacrimal caruncle are reported in elderly men. In the first case the tumour was ascribed to an accessory lacrimal gland. In the second the tumour was malignant and appeared to be a sarcoma of a vascular origin.

Pigmented Tumours. REESE, A. B. (1947). *Amer. J. Ophthal.*, 30, 537.

An exposition and classification of the pigmented tumours of the eye and its adnexa, which may be summarized according to the origin of the melanoblasts as follows:

1. Neurogenic melanoblasts
   (a) Schwann cells ⎫ Benign and malignant neuro-
   (b) Naevus cells ⎭ genic melanoma.
   (c) Melanoblasts of secondary optic vesicle giving rise to:
      (i) Tumours of pigment epithelium of retina or ciliary body: benign and malignant neurogenic melano-epithelioma.
      (ii) Tumours of muscle of iris: leiomyoma.
      (iii) Tumours of pigment epithelium of iris: dictyoma.
   (d) Melanoblasts of leptomeninges: melanoblastic meningiomata.
2. Ectodermal melanoblasts: pre-cancerous and cancerous melanosis of lids and conjunctiva.
3. Mesodermal melanoblasts of uvea: benign and malignant chromatogenic melanoma.

Fluorescein as an Agent in the Differentiation of Normal and Malignant Tissues. MOORE, G. E. (1947). *Science*, 106, 130.

The author injected 5 ml. of 20% sodium fluorescein intravenously into patients suspected of having malignant neoplasms, and examined the tissue with an ultraviolet lamp emitting rays at about 3,600 degrees. When the interval from injection to examination was between 3 and 8 hours a difference between normal and malignant tissues was observable. In patients subjected to laparo-

tomy for gastric carcinoma, implants of tumour tissue on the peritoneal surface were readily seen, because they fluoresced with a vivid yellow colour. When the tumour tissue was more than a few mm. below the surface no fluorescence was observed, presumably because of the failure of ultraviolet light to penetrate the tissue. Of 46 neoplasms of different organs examined by this technique, 31 exhibited a high degree of fluorescence, 6 only slight fluorescence, and 9 none at all. In each case malignancy was confirmed by histological examination. Carcinomata of the colon, stomach, and breast were less likely to fluoresce, but the best results were obtained with brain tumours. With subcortical lesions the method has been of particular value. Tumour tissue taken from suspected areas by aspiration-needle biopsies was readily recognized by its exaggerated fluorescence in ultra-violet light. A source of confusion in studying fluorescence of abdominal masses is introduced by the fact that areas of oedema and cyst formation may retain the dye for many hours. *R. J. Ludford.*

Meningeal Gliomatosis. A Study of Forty-two Cases. POLMETEER, F. E., and KERNOHAN, J. W. (1947). *Arch. Neurol. Psychiat., Chicago*, 57, 593.

The authors have collected 42 cases of "meningeal gliomatosis" over a period of 20 years. In 47.6% of cases the primary tumour was a medulloblastoma, in 14.3% glioblastoma multiforme, in 11.9% ependynoma or ependymoblastoma, in 11.9% oligodendroglioma or oligodendroblastoma, in 7.1% astrocytoma, in 4.8% retinoblastoma, and in 2.4% pinealoma. In the 42 cases reviewed there was no instance of metastasis outside the central nervous system. The paper includes a brief review of the subject of glial heteropia, first described by Wolbach in 1907.

Involvement of the Nervous System by Malignant Lymphoma. SPARLING, H. J., ADAMS, R. D., and PARKER, F. (1947). *Medicine, Baltimore*, 26, 285.

The authors classify malignant lymphomata into 6 types: (1) lymphocytoma, lymphocytic lymphosarcoma, and lymphatic leukaemia; (2) lymphoblastoma, lympho-blastic lymphosarcoma, and lymphatic leukaemia; (3) giant follicle lymphoma; (4) Hodgkin's disease; (5) reticulum-cell sarcoma; (6) plasmocytoma and myeloma. They report 19 cases of involvement of the central nervous system among 118 cases of lymphoma examined at the Mallory Institute of Pathology between 1930 and 1945. In all forms paraplegia due to invasion of the spinal epidural space by growth may occur, and it is the common neurological lesion in giant-celled lymphoma, Hodgkin's disease, reticulo-sarcoma, and plasmocytoma. The authors adduce evidence that the damage to the cord may be due to vascular disturbances. Hodgkin's disease and reticulosarcoma may, however, appear as intracerebral tumours. In leukaemia and lymphosarcoma there may be diffuse infiltration of the meninges and nerve roots, but the most frequent lesion is one or more cerebral haemorrhages. The authors give full clinical and pathological descriptions of the new cases and comment on those previously reported.

J. G. Greenfield.

On the Origin of Heparin. An Examination of the Heparin Content and the Specific Cytoplasmic Particles of Neoplastic Mast Cell. OLIVER, J., BLOOM, F., and MANGIERI, C. (1947). *J. exp. med.*, 86. 107.

The authors have estimated the heparin content of

two mast-cell tumours in dogs. The first contained 50 times, and the second 1.7 times, as much heparin, weight for weight, as normal dog's liver. The findings support the theory that the mast cells produce heparin.

The Examination of Serous Fluids by the Cell-block Technic. CHAPMAN, C. B., and WHALEN, E. J. (1947). *New Engl. J. Med.*, 237, 215.

This is an analysis of the results of cytological examination of pleural and peritoneal exudates by the method of sectioning a "false tissue" centrifuge deposit. At the Boston City Hospital during the last 15½ years 833 samples of fluid have been so examined. The material came from 666 patients, in 102 of whom positive results for new growth were obtained. Of 114 cases in which malignant disease was found at necropsy or biopsy, cell-block preparations were positive in 47. The diagnostic criteria were rather more stringent than those often employed elsewhere. For a positive diagnosis the authors require that fully or partly formed acini or sheets composed of cells showing definite evidence of anaplasia should be present. *D. H. Collins.*

Significance of the Cell Types of the Human Islets of Langerhans in Islet Function and Diabetes Mellitus. (Die Bedeutung der Zelltypen des menschlichen Inselapparats für Inselfunktion und Diabetes mellitus.) TERBRUGGEN, A. (1947). *Klin. Wschr.*, 24/25, 434.

Employing a technique similar to that used in animals by Lane and Bensley, the author has examined the number of α- and β-cells in the pancreas of normal and diabetic men. There is in diabetic subjects both a relative and an absolute reduction in the number of β-cells in the islets of Langerhans and this reduction seems to be responsible for the disease. The ratio of α-cells to β-cells in normal adults has been found to be from 1 : 3 to 1 : 5. In juvenile diabetic patients (those who died before the age of 40; 8 cases) the ratio was mostly from 1 : 1 to 1 : 2 and was in no case higher than 1 : 3. Results in diabetic subjects over 40 (18 cases) were not so clear. Here the results are complicated by other changes in the islets, especially hyaline degeneration and sclerosis, but in islets not hyalinized and not sclerosed the same diminution of β-cells has been observed. In hyperinsulinism a relative diminution of the β-cells has also been observed; this may be explained as an adaptation. In adenomata of the islands very few α-cells are seen. The β-cells obviously do not originate from the α-cells; the latter may represent a stage of rest or be regarded as a special kind of cell.

O. Neubauer.

The Histopathology of Acute Mumps Orchitis. GALL, E. A. (1947). *Amer. J. Path.*, 23, 637.

During an epidemic of mumps among adult males, orchidotomy was practised for acute mumps orchitis and biopsies of testis from 75 such cases were taken, the majority within 48 hours of onset and none later than the fifth day. Necropsy material was obtained from 1 patient who died of pulmonary embolism 11 days after the onset of orchitis. Stages of degeneration and inflammation are described. Complete atrophy is unusual, although permanent focal damage may result. Owing to the interval of 11 days between onset of orchitis and death, the lesions were more intense and the processes of repair became more apparent; fibrosis and hyaliniza-

tion of the lamina propria of the affected tubules were beginning, macrophages were more numerous, and polymorphs were scarce. Several groups of tubules showed actively proliferating normal epithelium.

Specimens of epididymis revealed interstitial changes similar to those in the testis. The epithelium seemed normal, but the lumina were filled with plugs of debris coming from the damaged tubules.

Dysontogenetic Pituitary Cysts. (Pituitary Cachexia in Childhood.) BARR, H. S. (1947). *Arch. Dis. Childh.*, 22, 118.

Two cases of pituitary cachexia in childhood were due to large colloid cysts of Rathke's cleft with pressure atrophy of the anterior lobes. The designation "dysontogenetic pituitary cysts" for this type is proposed. The reasons are pointed out why the designation "tumours of Rathke's pouch" for carniopharyngiomata is considered to be a misnomer. The pituitary cachexia was associated with syringomyelia in one case and with fibrocystic dystrophy of the pancreas in the other. The possibility of a causal link between the latter and the pituitary disease is discussed.—[From the author's summary.]

Hyperealcemia and Idiopathic Hyperplasia of the Parathyroid Glands in an Infant. PRATT, E. L., GEREN, B. B., and NEUHAUSER, E. B. D. (1947). *J. Pediat.*, 30, 388.

An infant was studied from the age of 15 weeks until its death at 10 months. The leading symptoms were weakness, hypotonia, lethargy, anorexia, and malnutrition. Investigations revealed a constantly high blood calcium with low urinary calcium excretion and deficient renal function. Radiographs of bones showed some hypocalcification, and biopsy revealed fibrosis of the marrow spaces.

At necropsy there was very slight enlargement of the parathyroids, which microscopically showed lack of stroma, great cellularity, and increase in chief cells and transitional water-clear cells. Some nephrocalcinosis and a calcium plaque in the aorta were seen, but no other metastatic calcification was observed. The bones showed osteitis fibrosa. No satisfactory explanation is offered for the apparently unique association of hypercalcaemia with chief-cell hyperplasia.

N. M. Jacoby.

Myelolipoma of the Adrenals. Report of Seven Cases. GIFFEN, H. K. (1947). *Amer. J. Path.*, 23, 613.

Seven examples of heterotopic development of bone marrow in the adrenal glands are described, 4 with yellow and 3 with red marrow; all were in middle-aged or elderly people, none of whom showed significant anaemia. The cause and histogenesis of the change are obscure.   *R. A. Willis.*

Polycystic Disease of the Kidney. A Review. LAMBERT, P. P. (1947). *Arch. Path.*, 44, 34.

It has been proved by serial sections that the cysts in neonatal cases do not communicate with the pelvis; these patients survive for only a short time. Although the adult type is clinically so different (in that the patient often lives to middle age), it has generally been assumed that the cysts were also closed in this type. Cysts in adult cases had not been studied in serial sections because of their size, but this has now been done in 5 adult cases with unexpected results.

Cysts are classified as: (1) Glomerular cysts: these have a normal glomerular tuft and granular content. They never communicate with the pelvis in either adult or neonatal cases. (2) Tubular cysts: these have a colloid content. They communicate with the convoluted tubules or loops of Henle, and in adult cases the nephrons thus affected generally communicate with the pelvis. (3) Excretory cysts, connected with the collecting tubules. All the above may be simple or complex. (4) Cysts of the calices.

The author produces evidence that the nephrons connected with tubular and excretory cysts function normally, thus accounting for survival of these cases into middle age. The author discredits previous theories of the genesis of the disease, and it is clear that non-union of ureteric tubules and nephrons will not account for the formation of cysts which communicate with open tubules.   *D. M. Pryce.*

Acute Liver Necrosis in Fulminating Infectious Hepatitis. TAYLOR, H. E. (1947). *Amer. J. clin. Path.*, 17, 314.

Four fatal cases of fulminating liver necrosis in infectious hepatitis, homologous serum jaundice, and arsenotherapy jaundice have been presented. The length of illness was 4 or 5 days. The essential lesion was an extremely rapid diffuse necrosis and disintegration of the parenchymal liver cells. No normal cells were found in the many blocks examined. There was an associated meningo-encephalitis in 2 cases.—[From the author's summary.]

The Pathology of Epidemic Hepatitis. MALLORY, T. B. (1947). *J. Amer. med. Ass.*, 134, 655.

Biopsies of liver from 160 patients in various stages of the disease, and necropsy material from 296 cases have been studied. Histological stages of the disease are described.

Studies of Gaucher Cells. (Etudes sur la "cellule de Gaucher.") FRANCO, S., and WOLMAN, M. (1947). *Schweiz. Z. Path. Bakt.*, 10, 621.

A staining method is described for the demonstration of keratin in the spleens of patients who died from Gaucher's disease. Frozen sections of formalin fixed tissue are treated with ether and acetone to remove the fat; after the sections have been exposed for 30 seconds to boiling water at pH 4 they are stained with Sudan III. The method is specific for cerebrosides, and in the cases described for keratin.—[From the author's summary.]

The Cutaneous Lesions in Acute Meningococcemia. A Clinical and Pathologic Study. HILL, W. R., and KINNEY, T. D. (1947). *J. Amer. med. Ass.*, 134, 513.

This paper describes the pathogenesis of the cutaneous lesions in acute meningococcaemia, through the stages of endothelial damage, inflammatory necrosis, and thrombosis. The first is responsible for the purpuric rash sometimes proceeding to local gangrene. In the early stages histological examination may show desquamation of vascular endothelial cells, which elsewhere may exhibit phagocytosis of cocci. Invasion by polymorphonuclear leucocytes follows, many cells displaying active phagocytosis. Platelet thrombi, becoming organized in the later stages, were most commonly found in deeper vessels of the cutis, though the superficial

capillaries were also involved. The stained sweat glands were abnormally pale, their cytoplasm appearing swollen or vacuolated, the result of interference with the blood supply of the gland. The hair, muscles, and nerves showed similar degenerative changes and focal necroses were seen in subcutaneous fat. Similar vascular lesions were found in many other organs and are by no means limited to the skin. The authors suggest that the formation of thrombi may cause thrombocytopenia and thus lead to purpura. They say that in 2 cases without purpura the platelet count was normal. Other workers, however, have found normal platelet counts in patients showing a purpuric rash. *R. H. D. Short.*

Transitory Diabetic Syndrome associated with Meningococcic Meningitis. Fox, M. J., Kuzma, J. F., and Washam, W. T. (1947). *Arch. int. Med.*, 79, 614.

In 233 cases of meningococcal meningitis, 1 in 4 showed transient diabetes mellitus. Fortunately, if the septicaemia is well and promptly treated the diabetic syndrome subsides. In 5 cases a wrong diagnosis was made, and the patient, who was in fact suffering from meningococcal septicaemia, was deemed to be in a state of diabetic coma and treated accordingly. During such a mistaken course of treatment irreparable damage may be done to the tissues and viscera. Seventeen cases were examined at necropsy, but the findings do not sustain the contention that glycosuria in meningitis may be due to damage to the pituitary as all the viscera, especially the liver, thyroid, spleen, pancreas, and adrenals, showed damage. *G. F. Walker.*

Histopathologic Changes Associated with Human Poliomyelitis. Scheinker, M. (1947). *Arch. Neurol. Psychiat., Chicago*, 57, 565.

The results of studying 6 cases of acute anterior poliomyelitis support the now generally held view that this disease of the central nervous sytem is not as focal as was formerly thought but can be widespread in its incidence. The author finds no direct parallelism between the neuronal destruction and the inflammatory reactions in the mesoderm. Cellular infiltration, for instance, is most intense in the medulla and midbrain, especially in the vicinity of the nucleus ambiguus, substantia nigra, and aqueduct, in all of which places neuronal destruction is slight compared with that in the anterior horns of the spinal cord. These cases, in that they exhibited massive inflammatory changes in the region of the vagal nuclei, tend to support the view that the virus may spread from the upper part of the alimentary tract to the brain through the fifth, seventh, ninth, and tenth cranial nerves. *W. H. McMenemy.*

A Clinico-Pathological Report of Unusual Cases of Chronic Encephalitis. Rosanoff, W. R. (1947). *J. Neurol. Neurosurg. Psychiat.*, 10, 65.

Three cases of encephalitis are described. All were insidious in onset and progressive over periods varying from 4 months to 5 years. They were characterized by intense inflammatory changes widely distributed throughout the brain. Case 1 was distinguished by a particularly severe involvement of the cerebral white matter which closely resembles the "leuco-encephalite sclerosante subaigue" of van Bogaert. The second had a positive iron reaction. In the third there was the combination of diffuse and focal cystic lesions characteristic of some of the extra-European types of epidemic encephalitis.— [From the author's summary.]

Deposition of Iron in Paraventricular Areas of the Human Brain in Hemochromatosis. Cammermeyer, J. (1947). *J. Neuropath. exp. Neurol.*, 6, 111.

The author studied the deposition of iron pigment in the brain in a long-standing case of haemochromatosis and compared his findings with the results of injections of vital dyes by Wislocki, King, and others. He concludes that the deposition of iron in the paraventricular areas of the brain is related to structural and functional peculiarities of the blood vessels in these areas. *J. G. Greenfield.*

Studies on Typhoid Pneumonia. Communication I. Pneumonia Types in Typhoid Fever (in Russian). Lazovsky, Y. M., Kudravtseva, E. N., and Melnik, E. G. (1947). *Klin. Med., Mosk.*, 25, 35.

The authors examined 35 cases of pneumonia in typhoid fever and concluded that although 15 were of the hypostatic type, 20 showed changes which they considered specific for typhoid pneumonia and which resembled the lesions found in the alimentary canal. Whole lobes were often involved, and pneumonia was bilateral in 13 cases.

Pulmonary Vascular Lesions in Silicosis and Related Pathologic Changes. Geever, E. F. (1947). *Amer. J. med. Sci.*, 214, 292.

An analysis is made of the vascular lesions in 43 unselected and consecutive cases of pulmonary silicosis. In 26 there was hypertrophy of the right ventricle attributable to the silicosis alone. Changes in the lung parenchyma are also described.

Lymphogranuloma Venereum. A Histologic Study of the Primary Lesion, Bubonulus, and Lymph Nodes in Cases Proved by Isolation of the Virus. Sheldon, W. H., and Heyman, A. (1947). *Amer. J. Path.*, 23, 653.

The authors suggest that the histological appearances of biopsy specimens are sufficiently typical to enable diagnosis to be made with reasonable certainty. Their observations are based on examination of material obtained from 12 cases of the disease in which the diagnosis had been confirmed by large numbers of tests, both clinical and laboratory. In 8 of these cases absolute proof was obtained by isolation and identification of the virus from material derived from the lesions.

The earliest lesion is the formation of foci of large mononuclear cells in the adventitia of the small blood vessels or, in the case of the lymph nodes, in the cortex just beneath the marginal sinus. The proliferation proceeds to involve all the coats of the blood vessels and eventually obliterates their lamina by compression, and *without* the vascular endothelial proliferation or thrombosis usually stated to take place. Similar changes affect the sinuses and small capillaries in the lymph nodes, so that small granulomata are produced. Their centres undergo ischaemic necrosis and numerous polymorphonuclear leucocytes appear. Thus small abscesses are formed which by fusion form larger ones and, if the lesion be near the skin, as in the case of the primary lesion particularly, the epidermis breaks down and an ulcer is formed. Peripherally the abscess is surrounded by a ring of mononuclear cells, outside which a few giant cells may be found together with plasma cells and

lymphocytes in small numbers and a very occasional eosinophil leucocyte. Fibrosis was scarcely noticeable in the acute stages, and was present only to a small degree in the node removed from the patient in whom the disease had been cured. The authors found the eosinophilic intracytoplasmic inclusions, or "gamma bodies," only after necrosis had taken place, and believe that they are merely debris phagocytosed by the mononuclear cells and bear no relation to the virus of lymphogranuloma. No elementary bodies were found.

Temporal or Giant-Cell Arteritis. ROBERTSON, K. (1947). *Brit. med. J.*, 2, 168.

Details are given of 4 cases of temporal arteritis met with in a period of a little more than a year, 2 of which were proven by biopsy. The patients were aged 65 (2), 73, and 65. In 3 cases the erythrocyte sedimentation rate was high for a considerable time. The blood urea is normal. Progressive secondary anaemia and mild leucocytosis were present. The disease is self-limited and rarely fatal, and treatment is entirely symptomatic. It differs from periarteritis nodosa in that the latter attacks younger people; the visceral vessels in periarteritis nodosa suffer severely, the blood urea is almost always raised, and the mortality from the disease is high.

<div align="right">S. Oram.</div>

Ostropetrosis : Albers-Schonberg Disease (Marble Bones). Report of a Case and Morphologic Study. PINES, B., and LEDERER, M. (1947). *Amer. J. Path.*, 23, 755.

The authors review the literature and present details of the necropsy and histology of the bones in an infant. The original paper should be consulted.

Nodular Inflammatory and Degenerative Lesions of Muscles from Four Hundred and Fifty Autopsies. CLAWSON, B. J., NOBLE, J. F., and LUFKIN, N. H. (1947). *Arch. Path.*, 43, 579.

In small biopsies of skeletal muscle in rheumatoid arthritis Steiner *et al.* found chronic inflammatory foci composed mainly of lymphocytes in each of 9 cases. They also noted various degenerative lesions in the muscle fibres. They found no similar lesions in a control series of 196 necropsy specimens, and considered them specific and indicative of an infective aetiology. The present authors found lymphocytic foci in 39% and degenerative lesions in 25% of 44 clinical cases of rheumatoid arthritis. They noted lymphocytic foci in 26% and degenerative lesions in 42% of 450 controls. The incidence increased with age. The muscles most commonly affected were the diaphragm and sacro-spinalis. Although more common in association with rheumatoid arthritis, the lymphocytic infiltrations are not specific.

<div align="right">D. M. Pryce.</div>

Deaths Following Use of Abortifacient Paste. Report of Two Cases. KULKA, W. (1947). *Amer. J. clin. Path.*, 17, 723.

The necropsy findings are described in 2 patients in whom death followed the injection of an abortifacient paste. In 1, death occurred 2½ months after the attempted abortion. Necrosis of the uterine wall, parametrial abscesses, and generalized peritonitis were found. The second patient died suddenly of pulmonary embolism within a few hours after an attempt to cause abortion by intrauterine injection of the paste. The emboli were composed of fatty material and particles from the damaged chorionic villi. There was necrosis of some of the villi and vessels of the uterine wall but no apparent changes in the embryo or the embryonic sac. The presence of a pasty material was demonstrated by the technique used as a routine for the demonstration of fat but the staining reaction was different from that of fat.— [Author's summary.]

Sudden Deaths of Infants Allegedly Due to Mechanical Suffocation. WERNE, J., and GARROW, I. (1947). *Amer. J. publ. Hlth*, 37, 675.

During the past 15 years 167 consecutive cases of infants ordinarily certified under the heading of "accidental mechanical suffocation" have been investigated by the authors. In no case did they find that an infant had been suffocated. In 43 cases necropsy alone was sufficient to determine that death was due to a natural cause, mainly upper respiratory tract infection. In the remaining 124 cases histological study showed that fulminating respiratory disease was the most likely explanation for death in all except 15, in 6 of which no tissues were available for microscopical examination. An examination was made of 67 other infants who had died suddenly in circumstances in which there could be no possible allegation of smothering. In these, acute respiratory disease was the usual cause of death.

OBITUARY

EDWARD FFOLLIOTT CREED

It is with sorrow that we have to record the sudden death of Edward ffolliott Creed at the age of 54 during his convalescence from a severe illness. He was one of the original members of the Association of Clinical Pathologists. He had succeeded W. D'Este Emery as director to the Pathological Department at King's College Hospital in 1921. There had been several months' delay in filling the post, perhaps because of doubts in the minds of his colleagues due to his relative youth and inexperience. These doubts were soon set at rest and the Department flourished under his care.

Creed was largely responsible for what was, in the early nineteen-twenties, an innovation in teaching hospitals—namely, the creation of a post of resident assistant clinical pathologist. His intention was probably twofold: first, to introduce the budding physician or surgeon to a working knowledge of laboratory methods in clinical medicine, and help him not only to understand clinical medicine the better but also, and perhaps more important, to appreciate the value and limitations of laboratory methods ; secondly, to provide the early training ground for the clinical pathologist of the future. This was a very necessary provision, because up till then almost the only way for one who wished to become a clinical pathologist was to enter a university pathological laboratory as a junior demonstrator and go through a training which, though of great value educationally and scientifically, often failed to provide all that was needed for one who would be in charge of a clinical laboratory. The resident pathologist also learnt to bridge the gap between bedside and laboratory, on the one hand helping his colleagues the house physician and house surgeon to use the laboratory more efficiently and less wastefully ; and on the other, keeping the laboratory closely in touch with what was going on in the wards. Creed felt strongly the constant need for this close association, and resisted all measures which threatened it, such as the siting of laboratories at distances from the wards. *Pari passu* he was not attracted by postal pathology,

EDWARD FFOLLIOTT CREED

and he tolerated it only because of the exigencies of the time.

H. A. Osborn was the first, Terence East the second holder of the new post. I was the third, and there have since been a succession of trainees who are now scattered over the country, many of them running laboratories ; some, practising in other fields, now

know that their spell in clinical pathology under his guidance was time well spent.

To one closely in touch with him over the last quarter of a century, an outstanding feature in his character was his humility and tolerance, shown even to the callow and the ignorant. He remained free from the accretions of dogma and self-assurance which are apt to clog the minds of teachers in their fifth or sixth decades. His humility was genuine, and prevented him from publishing work which ought to have been published long since—for example, his careful and critical elaboration and assessment of the technique of blood fragility estimation, which he did not publish for some fifteen years. His humility was not due to any lack of critical faculty ; he was quick in finding weak spots in an argument. Some of us can recall the quiet way in which after a meeting he could on occasions tear to pieces an elaborate and convincing thesis.

He was a pupil of Georges Dreyer, of Oxford, and was essentially a clinical pathologist. His interest always lay in the practical application of pathology to medicine. He was too occupied in perfecting techniques for routine work to tackle more basic problems. He upheld the tradition that the pathologist should be constantly at the bedside, and as his interest lay in clinical problems his heart was in his work.

A careful and patient teacher to the many, to the few who knew him well his loss will be keener and more personal. They will miss a delightful and sympathetic companion, perhaps a trifle reserved, but one whose conversation, full of wisdom, was lit by frequent shafts of whimsical humour.

C. H. WHITTLE.

We regret to record the death of Professor James McIntosh, Director of the Bland-Sutton Institute of Pathology, Middlesex Hospital, and Professor of Pathology in the University of London.

GIANT-CELL OR TEMPORAL ARTERITIS: A REVIEW

BY

C. V. HARRISON

Postgraduate Medical School of London

Historical

The first case of what is now known as temporal, cranial, or giant-cell arteritis was published in 1890 by Jonathan Hutchinson. He described how the 80-year-old father of a London Hospital beadle came to him complaining that he could not put his hat on because of painful swellings in his temples. These proved to be red, inflamed, swollen temporal arteries which subsequently lost their pulsations and were left as hard cords after the condition healed. The man recovered and lived for a number of years. The next case was recorded in 1930 by Schmidt. His patient showed the characteristic clinical picture and is of interest because he developed an intracranial aneurysm, a complication which has since been recorded in one other case (Andersen, 1947a). In 1932, Horton, Magath and Brown, of the Mayo Clinic, described two cases and, being presumably unaware of Hutchinson's and Schmidt's cases, regarded them as examples of a new disease and gave the condition the name of "temporal arteritis." They believed that the disease was limited to these arteries and followed a benign course free from complications. In 1934 and 1937 they recorded five further cases. In 1934 Paviot and others recorded the first case from France, but they did not mention any previously published cases and do not appear to have recognized the significance of their case. It is, however, interesting to note that their patient showed signs of involvement of the carotids as well as of the temporal arteries. In 1935 Barnard described the post mortem findings in a woman who had shown some of the signs of temporal arteritis and who had become blind. The temporal arteries were not described either in the clinical or autopsy account, but the description of the histology in the carotid and coronary arteries strongly suggests that this was an example of what is now called "giant-cell arteritis." In 1938 Jennings described the first two British cases corresponding to the descriptions of Horton and others, and he was the first to recognize blindness

as a complication of this disease. Since then cases have been recorded from U.S.A., Britain, France, and Scandinavia; the total of published cases to date is 75.

Nomenclature

This disease was first called "temporal arteritis" by Horton and others in the belief that it was localized in these arteries. As further cases were recorded it became apparent that this was far from true. Kilbourne and Wolff (1946), being impressed by the frequency of involvement of the cerebral and retinal vessels, suggested the term "cranial arteritis," and this was adopted by Curtis (1946) and Meyers and Lord (1948). A few writers have included the eponym "Horton's disease," but this has fortunately not been followed. Gilmour (1941) introduced the title "giant-cell arteritis" and this was followed by Robertson (1947).

None of these titles is satisfactory. The disease is far too widespread in its distribution for either "temporal" or "cranial" to be accurate, and giant cells have not been demonstrated in all cases. Nevertheless, on the score of simple accuracy, "giant-cell arteritis" is certainly the best title of the three. There is, however, one point in favour of retaining Horton's original term, and that is that it draws attention to the dominating and most diagnostic clinical lesion. It seems likely that the terms "temporal" and "giant-cell" will flourish side by side.

Clinical Characteristics

(For summary of findings in the reported cases see Table.)

Sex.—The early reports included a high proportion of women, but since then more cases have been reported in men, and the figures now are 35 men to 40 women, which suggests that there is probably no real sex bias.

Age.—Giant-cell arteritis is a disease of the elderly, the mean age of the 75 recorded cases being 65.3 years. The majority have been over 60,

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and all but two over 50. The exceptions were women of 22 (Meyers and Lord, 1948) and 23 (Gilmour, 1941). Gilmour's case was clinically atypical, but the histology of the vessels appears to be characteristic; the case of Meyers and Lord was a typical one clinically, though the biopsy, taken during a recurrent attack, showed only a healed arterial lesion.

Onset.—The disease begins as a vague illness, with malaise, anorexia, bodily aches, sweats, and often fever. This prodromal stage may last from a few days (Bain, 1938 ; Dick and Freeman, 1940) up to eighteen months (Cooke and others, 1946; Andersen, 1947), but usually lasts about one or two months. At the end of this time the patient develops pain in the head. This is usually referred to the temple, and is severe, often enough to interfere with sleep. In many cases it is described as coming in attacks of great violence with a dull ache between the attacks. The pain usually resists ordinary analgesics and may not be fully controlled by morphia. Soon after the onset of pains in the head the temporal arteries become extremely tender; they usually appear as tortuous nodular cords covered by a red, inflamed skin. In the early stages pulsation is still palpable but this is usually lost during the height of the disease, though it may return later (Profant, 1944). Many of the clinical reports refer to the arteries as thrombosed; but thrombosis is unusual in the descriptions of the histology, and the hard, cordlike texture is probably due to a filling of the lumen by intimal proliferation rather than by clot. Enlarged cervical glands have been noted by MacDonald and Moser, 1937, Dick and Freeman, 1940, Hoyt and others, 1941, and Scott and Maxwell, 1941. The associated clinical findings are usually few. The patients are ill out of proportion to the physical signs; they may show mental changes (Sprague and MacKenzie, 1940 : Schaefer and Sanders, 1942 ; Broch and Ytrehus, 1946), but examination of the central nervous system usually reveals no physical signs or only equivocal and transitory ones. The blood pressure in these patients is most often normal, and the number of cases with hypertension is probably no greater than would be expected in a control population of this age. Similarly the signs of generalized vascular sclerosis are no greater than is likely in a random group of elderly people.

Laboratory findings.—Almost every known laboratory examination has been performed on these patients, and particularly tests for bacterial agglutination. These, and also the Wassermann reaction, have been consistently negative. Blood

examination alone has given any reasonably constant results. Anaemia of hypochromic type (60 to 85 per cent haemoglobin) has been recorded in twenty-nine cases ; it is rarely severe. A leucocyte count has been recorded in thirty-six cases and has varied from normal up to 24,500 per c.mm. of blood (Profant, 1944), the mean figure being 10,500 per c.mm. The blood sedimentation rate has been recorded as raised in twenty-one cases, and whilst the variety of methods used precludes any mean figure, the results recorded have mostly indicated a very abnormal figure. This raised sedimentation rate persists throughout the course of the clinical illness, and may continue for some time after symptomatic recovery. The cerebrospinal fluid has been examined in a number of cases and has sometimes shown a rise of protein and cells, but these findings appear to be inconsistent. Blood cultures have been consistently negative, as have electrocardiograms and radiographs.

Course.—The disease runs a slow course over many months. In the sixty-two cases in which the duration is stated, it has varied from about eight weeks to thirty months (Cooke and others, 1946). The mean for the stated figures is 7.2 months, but this is almost certainly an underestimate because in a significant number of cases the patient has not fully recovered at the end of the stated time. As a rule the severity of the symptoms diminishes and the patient is discharged from hospital without major symptoms but often still far from fit, and final recovery to normal health may be delayed for a number of months.

Prognosis.—In their early papers Horton and others stated that the disease ran a benign course free from mortality and complications. This has since proved to be incorrect. Of the seventy-five recorded cases, fifty-nine have recovered and sixteen have died ; but it is difficult to assess how many of these died as a result of the disease. This will be discussed later.

Treatment.—No effective form of therapy has yet been discovered. Individual cases have responded to the most diverse forms of treatment. The case of Paviot and others (1934) responded dramatically to salicylates, and those of Justin-Besançon and others, and of Lucien and others responded to sulphonamides; but many other cases have failed to respond either to sulphonamides or penicillin. The one form of therapy which has given the most constant symptomatic relief has been biopsy of the temporal artery. This had been recorded in twenty-one cases. In only two cases has it been specifically stated that biopsy failed to

afford relief (Sprague and MacKenzie, 1940; Dantes, 1946). The relief is symptomatic, does not seem to affect the course of the disease, and may be due to the coincidental cutting of perivascular nerves. Such nerves have been noted in the fibrosed adventitia by Lucien and others (1939) and by the present author.

Complications.—The commonest and most serious complication is involvement of the eyes. This had been noted in twenty-five out of seventy-five cases, and in fifteen of these there has been permanent blindness of one (eight cases) or both eyes (seven cases). In the majority of cases the ophthalmoscopic findings have been trivial compared with the loss of function, and it is generally believed that the blindness is due to retinal ischaemia from involvement of the vessels behind the globe and out of range of ophthalmoscopic examination. Johnson and others (1943) suggested that this may be due to vascular continuity between the temporal and ophthalmic arteries via the lachrymal branch of the former, but since we now know that the disease is, in fact, widespread and involves many vessels, it is unnecessary to postulate vascular continuity.

The second most important complication is cerebral involvement. The cases of Sprague and MacKenzie (1940), Schaefer and Sanders (1942), and Broch and Ytrehus (Case 3, 1946) showed clinical evidence of cerebral damage but recovered. The cases of Gilmour (Cases 2 and 3, 1941) and Cooke and others (Cases 3 and 6, 1946) died of cerebral ischaemia, confirmed at autopsy. The cases of Chasnoff and Vorzimer (1944), Cooke and others (Case 1, 1946), and Curtis (1946) died from cerebral causes but autopsy was not performed. In addition, the cases of Schmidt (1930) and Andersen (1947a) developed signs of intracranial aneurysm. From the records of the cases which came to autopsy there is little doubt that the cerebral signs and symptoms are due to involvement of the cranial vessels and are due to ischaemia. They may be transitory or they may be fatal, but generally they do not seem to leave any sequelae if the patient recovers; though there seems to be no reason why this should not occur. The two cases of Sjövall and Windblad (1944) both developed a crippling arthritis, but they are the only two recorded cases to do so and it is possible that they represent a coincidence rather than a complication. Robertson's (1947) first case developed gangrene of the leg, but again that may have been a coincidence due to atheroma. It is certainly significant that, in a disease which causes gross vascular narrowing and affects medium-sized arteries, there is no ischaemic gangrene of the limbs.

Fatal cases and the extent of the disease.—The cases which have died are of sufficient interest to justify individual consideration. The first was that of Barnard (1935). This woman had been ill for seven months with an apparently undiagnosed condition which was characterized by headache and the loss of sight in one eye. She died of erysipelas, and at routine autopsy changes were noted in the internal carotid and coronary arteries. These lesions were characterized by medial necrosis and cellular infiltration, including giant cells, and were interpreted as tuberculous though no bacilli were found. In the light of the numerous cases since recorded it seems highly probable that this was an example of giant-cell arteritis in which the involvement of the temporal vessels was minimal. A similar case has been recorded by Andersen (1947b).

Sproul and Hawthorne (1937) recorded two cases neither of whom showed any significant signs of temporal arteritis and both of whom died, one of septic kidney and the other of cardiac infarction. One case showed histological lesions in the aorta and iliac artery and the other in the aorta, iliac, and carotid arteries. The descriptions strongly suggest that in these cases the arterial lesion was indistinguishable from that of giant-cell arteritis, though the temporal arteries were not apparently involved clinically and were not examined at autopsy. The authors did not regard their cases as being examples of giant-cell arteritis, and Sproul does not refer to this publication in a later paper (1942), in which he records the autopsy findings in a classical case of giant-cell arteritis who died of ischaemic heart disease. In the latter case there were typical and severe lesions in the aorta, innominate, subclavian, pulmonary, coeliac, mesenteric, renal, and iliac arteries (the temporal arteries were not examined at autopsy though they were clinically typically affected during life).

Gilmour (1941) recorded four autopsy cases. The first was of a woman of only 23 who had been ill for six months with giddiness and hot sweats after an influenza-like onset and who died of a ruptured subclavian aneurysm. Clinically the case was atypical, but the histology of the aorta, internal, and external carotid and subclavian arteries was exactly similar to the published descriptions of classical cases and it seems probable that this was a true case. The age is quite unusual, but a clinically typical case has been reported in a woman of 22, by Meyers and Lord (1948). Gilmour's second case, in a woman of 59,

TABLE

RECORD OF PUBLISHED CASES OF GIANT-CELL ARTERITIS

No.	Authors	Case Nos.	Year	Sex	Age	Duration	Recovered or died	Biopsy	Autopsy	Eye symptoms or signs	Fever	Illness	B.S.R.	W.B.C.	Bodily pains	Anaemia	Vessels affected	Relieved by biopsy	Comments
1	Hutchinson		1890	M	80	?	R	–	–		–	–		3,900	?	+	Both temp.		Intracranial aneurysm
2	Schmidt		1930	F	69	8/12	R	+	–	Scotoma	++++	++++		13,700	+		Both temp.		
3	Horton et al.	1	1932	F	55	22/12	R	++	–			++	+	10,600		+	Both temp.		Associated with active rheumatism.
4	Paviot et al.	1	1932	M	68	2/12	R	–	–	Unequal pupils							Both temp., both carotid.	+	
5	,,	2	1934	M	61	3/12	R		–								Carotid and coron.		
6	Barnard		1935	F	63	7/12	D	++	+	L. blind		+++		8,600	+	+	Both temp.		Glands +. Root abscess.
7	MacDonald and Moser		1937	F	60	6/12	R	+	–	–		+					Both temp.		
8	Horton and Magath	1	1937	F	69		R	+	–	–		+					Both temp.		
9	,,	2	1937	M	75		R	+	–	–		+					Both temp.		
10	,,	3	1937	F	72		R		–	–		+					Both temp.		
11	,,	4	1937	M	65	6/12	R		–	–		+					Both temp.		
12	,,	5	1937	F	68	2/12	D		++	–		–		13,000			Both temp.		Died of septic kidney.
13	,,	6	1937	M	57		D			–				9,100			Aorta and iliac.		Died of cardiac infarct.
14	Sprout and Hawthorne	1	1937	F	76		D			–				12,500			Aorta, iliac, carotid.		
15	,,	2	1937	M	50		D			–				3,500			Both temp.		
16	Jennings	1	1938	F	66	6/12	R	+	–	L. blind	+++	+++++	+	13,100	+	++	Both temp.		Glands +. pneumonia. Followed
17	,,	2	1938	F	72		R	–	–	–	+	+					Both temp.	+	Tonsils Glands +. inflamed.
18	Bain		1938	F	71	10/52	R	++	–	–		++++					Both temp. and occipital.		
19	Thévenard		1939	M	66	9/12+	R	++	–	–		++					Temp., pariet., and P. auric.		
20	Lucien et al.		1939	F	79		R			–		+					Both temp.		
21	Dick and Freeman	1	1940	F	65	3/12	R	+	–	L. reduced field	+	+	÷		+		Temp., pariet., and occipital.	+	
22	,,	2	1940	F	76	13/12	R	–	–	Blurred vision	+	+					Both temp.		
23	Bowers		1940	F	65	6/12	R	++++	–	–	++++	++++	++	12,900	+	+++	Temp. and occipital	+|	Became mentally sluggish.
24	Sprague and MacKenzie		1940	M	66	6/12+	R	+++	–	–					+++		Both temp.	+	
25	Hines		1941	F	69	7/52+	R		–	–				20,000			R. temp. and radial.	+	Glands +. Inflamed fauces.
26	Hoyt et al.	1	1941	F	58		R		–	–	+	+++	+	14,000	+	+	Both temp.	+	Glands +.
27	,,	2	1941	F	61	7/52+	R		–	–	+	+++	÷		++	+	Both temp.		Glands +.
28	,,	3	1941	M	67	3/12+	R	–	+	L. blind	–	++					Temp., facial, and carotid.	+	Glands +.
29	Scott and Maxwell		1941	F	70	6/12+	R	–	+	–	+	+		10,300			Aorta, carotid, and subcl.		
30	Gilmour	1	1941	F	23	6/12	D	+	++	–	?	+++			+++		Aorta, carotid, cerebral.		Died of ruptured subclavian aneurysm.
31	,,	2	1941	F	59	5/12	D	–	++	–		÷					Aorta, carotid.		Cerebral infarct.
32	,,	3	1941	M	63	9/12	D	+|	+	Failing vision	–|	++	+|	4,000	+|	+	Aorta, innom., subcl., carotid, and iliac.		Cerebral infarct. Post-operative death.
33	,,	4	1941	F	64	26/12	D			–									Healed arteritis.
34	Sproul		1942	M	68	8/52	D	–	+	–	–	+	+	17,000	–	–	Temp. and others.		Many major vessels affected.
35	Plaut		1942	F	63	10/52	R	+|	–	Ptosis	+++	+++	+	9,700	+|	+|	Both temp.		Delirious. C.S.F. changes
36	Schaefer and Sanders		1942	F	62	3/12	R	–	–	L. blind	+	+				+	R. temp.		
37	Oldberg		1942	M	64	6/12	R	++	–	Both blind							Both temp.		
38	Murphy		1942	F	71	6/12+	R	–	–	One blind							Both temp.		
39	Johnson et al.	1	1943	M	61	3/52+	R	–	–	R. blind							Both temp.		
40	,,	2	1943	F	75		R	–	–	–							Both temp.		
41	,,	3	1943	F	80		R	–	–	–							Temp.–		
42	,,	4	1943	F	76	4/12+	R	–	–	–	+	+				+|	Both temp.		

TABLE—*continued.*

No.	Authors	Case Nos.	Year	Sex	Age	Duration	Recovered or died	Biopsy	Autopsy	Eye symptoms or sight	Fever	Illness	B.S.R.	W.B.C.	Bodily pains	Anaemia	Vessels affected	Relieved by biopsy	Comments
43	Brown and Hampson		1944	M	61	6/12 +	R	+	−	−	−	+	+	9,200	+	+	Temp. and occipital.		
44	Channou and Vorzimer		1944	F	63	14/12	D	+	+	−	+	+	+		+	+	Temp. and internal.	+	Died in coma 3/12 after biopsy. P.M. details not published.
45	Profant		1944	F	76	12/12	R	−	−	−	−	+++		24,500			R. temp.		Flare up after tooth extn. Pulsations reappeared.
46	Sjövall and Windblad	1	1944	M	61	2/12	R	−	−	−	−	+++	÷	12,000			Both temp.	+	Developed arteritis.
47		2	1944	M	62	12/12	R	+	−	−	−	+++	÷	5,900			Both temp.	+	Developed arteritis.
48		2	1945	M	73	9/12	R	+	−	−	−	+++		7,100			Both temp.	+	Developed arteritis.
49	Shanton and Solomon		1945			12/52	R	+	−	−	Both blind		÷			+		Temp. and ? occipital.		
50	Cooke et al.	1	1946	M	66	5/12	D	+	−	−	L. blind	−	÷	+	11,800			Both temp. and ? limb.	+	Died cerebral death 6/52 after discharge. No P.M.
51	" "	2;3	1946	M	66	6/12 +	R	÷	+	−	Both blind	−	÷÷	+	10,700	+	++	Both temp., aorta.	++	Died of cerebral damage.
52	" "	2;3	1946	F	69	30/12	D	−	−	−	Both blind	−	−		5,100	++		and femoral.		
53	" "	4;5	1946	M	71	6/12	R	+	−	−	Vision impaired	−	−		8,400	−	+	Both temp.	+	Had second attack. ? limb arteries involved.
54	" "	4;5	1946	M	69	12/12	R	−	−	−		−	−		10,000			Temp. and occipital.		
55	" "	6	1946	F	73	14/12	D	+	−	−	Both blind	−	−		8,300	+	+	Temp. and radial, and others.	+	Cerebral death. Many int. arteries affected.
56	" "	7	1946	F	68	12/12 +	D	−	−	−		−	−		13,200	+	+	Temp. and occipital.	+	
57	Hirsch and Yrichon		1946	M	69	5/12	D	−	−	−	Squint	+	+	÷		+	+÷−	Both temp.		Died 2/12 later of haematemesis. No P.M.
58	" "		1946	M	76	12/12	R	+	−	−	−	÷	÷	+		+	÷−+	Both temp.	+	Mental changes.
59	" "		1946	F	67	6/12	R	+	−	−	−	÷	÷	÷÷		+	+	Both temp.	+	Cerebral death. No P.M.
60	" "		1946	M	74	12/12	R	+	−	−	Both blind	÷	−			+		Both temp.	+	Dorsalis pedis involved.
61	Curtis		1946	M	71	4/12	R	−	−	−	−	÷	+	+	13,600	+	÷	Temp. and leg.	+	Died of cardiac infarct 3/12 later. No P.M.
62	Dimes		1946	F	55	19/52	D	+	−	−	Retinal exudate	÷	+	+		+		Temp. and limb.	+	Left with limb pains and intracranial aneurysm.
63	Gordon and Thurber		1946	M	68	3/12	R	−	−	−	−	÷	+			+		Both temp.	÷	Temp. arteries less affected than others.
64	Kilbourne and Wolff		1947a	M	54	24/12	R	+	−	−	Glaucoma	−	−	÷	7,300	+	+	Both temp.	÷	
65	Andersen		1947b	M	63	5/12	R	÷	−	−	−	+	+	+	4,700	+	+	Temp. and leg.	+	Leg gangrene, ? cause.
66	Andersen	1	1947	M	65	12/12	R	+	−	−	−	−	−	+	6,600	+	+	Temp., facial, occ. and leg.	−	Died P.G.U. No P.M. Thrombus in subclavian.
67	Robertson	2	1947	M	65	2/12	R	÷	+	−	Both blind	÷	+	+	9,000	+	+	Temp., facial, and occip.	+	? similar attack 3 years before.
68	" "	3	1947	F	73	?	R	−	−	−	−	÷	−			+		Temp., facial, and occip.		
69	" "	4	1947	M	67	?	D	−	−	−	−	−	−			+		Temp. and occipital.		
70	" "		1948	F	70	4/12	R	−	−	−	−	÷	÷	+	10,500	+		Temp., occip., and facial.	+	Biopsy in recurrent attack, healing lesion.
71	Austin-Besançon et al.		1948	F	72	3/12	R	÷	−	−	−	+	÷	+		+	÷	L. temp.	÷	
72	Meyers and Lord	1;2	1948	M	72	15/52	R	÷	−	−	−	÷	÷	−	12,800	+	÷	Both temp.	÷÷	
73	Cohen and Harrison	1;2	1948	M	65	8/52	D	÷	−	−	−		−	−		+		Both temp.	÷÷	Died 6/12 later from cardiac infarct. No P.M.
74	" "	1	1948	M	69	21/52	R	−	−	−	L. blind	−	−		6,200	+	−	Both temp.	÷	

had a five months' illness starting like influenza and characterized by temporal pain and noises in the head. She died in coma and at autopsy showed thrombosis of the internal carotid artery, and giant-cell arteritis of it and of the aorta. Gilmour's third case, a man of 63, had a nine months' illness with headache, mental signs, and failing vision. He too died in coma and at autopsy showed thrombosis of the internal carotid arteries and giant-cell arteritis of the aorta and of the external and internal carotid arteries. In Gilmour's fourth case, a woman of 64 suffered an ill-defined disease for twenty-six months and never showed any indication of temporal arteritis. She died of enteritis and amyloid kidneys, but at autopsy the aorta, innominate, common carotid, subclavian, and iliac arteries showed what the author interpreted as the healing stage of giant-cell arteritis. Chasnoff and Vorzimer (1944) record a case with typical histological appearances in the biopsy specimen of the temporal artery. Three months after discharge the patient returned to hospital in coma and died. The authors stated that at autopsy other internal arteries were involved and that details were to be published later by Mahoney and Hall, but no such publication can be traced.

Cooke and others (1946), in a series of seven typical cases of temporal arteritis, record three that died. Their first patient died from cerebral causes six months after discharge, and no autopsy was performed. Their second died from cerebral causes, and giant-cell arteritis was found in the aorta and in the femoral and mesenteric arteries. Their third also died from cerebral causes, and giant-cell arteritis was found in the aorta and in the superior mesenteric, femoral, radial, and retinal arteries. Broch and Ytrehus (quoted by Andersen, 1947a) record a typical case in which the patient died of haematemesis two months after discharge from hospital, but no autopsy was performed. Curtis (1946) described a patient with typical histological appearances in the biopsy specimen of the temporal artery who was readmitted in coma eighteen days after discharge and died, but no autopsy was carried out. Kilbourne and Wolff (1946) report a patient with typical biopsy findings who died of cardiac infarction three months later ; no autopsy was carried out. Robertson (1947) recorded a clinically typical patient who died a month later of a perforated gastric ulcer. At autopsy the subclavian artery was thrombosed but, unfortunately, no sections were taken.

The inclusion of some of these sixteen cases is open to criticism on the ground that as they did not show the clinical signs of temporal arteritis

during life, therefore the arterial disease found at autopsy is something different. Dantes (1946) has denied the authenticity of the cases of Barnard, Gilmour, and Sproul and Hawthorne. In the author's opinion this criticism is not justified for the following reasons. There is undeniable clinical and autopsy evidence that temporal arteritis is a widespread vascular disease. The clinical syndrome on which the diagnosis depends is the result of involvement of the superficial temporal arteries, and if these are excised for biopsy one of the dominant symptoms (localized head pain) usually disappears. If, therefore, a case of " temporal arteritis " should occur in which the temporal arteries were not involved the only really diagnostic clinical features would be lacking. In the cases recorded by Cooke and others, Sproul, and Chasnoff and Vorzimer the diagnosis was established clinically and was beyond question, and in these the larger internal arteries were involved. In the cases of Barnard, Gilmour, and Sproul and Hawthorne, the internal arteries showed apparently similar lesions, but the temporal arteries were not noted clinically or examined histologically and are, therefore, presumed not to have been involved. Nevertheless, it seems reasonable to assume that these were true cases and that in them the temporal arteries were either not involved or involved so slightly that they were overlooked.

These sixteen cases may give an exaggerated impression of the fatality rate in temporal arteritis. The cases of Barnard, Sproul and Hawthorne, and Gilmour (Case 4) did not die of their arterial disease. The cases of Sproul, Chasnoff and Vorzimer, Broch and Ytrehus, Curtis, Kilbourne and Wolff, and Robertson may have died of other diseases and not directly of the arteritis. Nevertheless, in the light of the first three cases of Gilmour and the second and third cases of Cooke and others, there is no doubt that temporal arteritis can prove fatal by cerebral involvement and it seems likely that it may also be fatal by involving other vital arteries.

These autopsy cases also illustrate the widespread nature of the disease, a point which has been stressed by clinical observers. Giant-cell arteritis is a disease of the larger arteries. The characteristic lesions have been described microscopically in the aorta nine times, in the carotid arteries six times, in the iliac arteries four times, in the mesenteric and subclavian arteries three times, in the innominate and femoral arteries twice, and in the pulmonary, coronary, coeliac, renal, radial, and retinal arteries once each. Most of these are arteries which are readily accessible dur-

ing the course of a routine autopsy, and the implication is that the disease is likely to affect any of the elastic and larger muscular arteries. It seems highly probable that other, less accessible, arteries were affected in these cases but were not examined.

Another point which emerges from these autopsy reports is the irregular distribution of the lesions. In most cases a number of vessels other than those listed above were examined and found unaffected. A further point is that the disease is apparently limited to arteries; the only reference to veins is made by Cooke and others, who state that the femoral vein was involved in one of their autopsy cases. It is, of course, possible that minor grades of venous involvement might be found if veins were systematically examined, but it is unlikely that the disease affects them severely or the naked-eye changes would have been noted. Apart from the larger arteries noted at autopsy there is clinical evidence that other arteries than the temporal are involved, especially the palpable vessels of the head. The vessels noted clinically are as follows: occipital artery (Lucien and others; Bowers; Brown and Hampson; Shannon and Solomon; Cooke and others; Robertson; Justin-Besançon and others); facial artery (Scott and Maxwell; Robertson; Justin-Besançon and others); carotid artery (Paviot and others; Scott and Maxwell); parietal artery (Dick and Freeman); posterior auricular artery (Dick and Freeman); radial artery (Hines); dorsalis pedis (Dantes).

Pathology

The lesions in the temporal arteries.—The following account of the morphology of the lesions is based partly on the published reports and partly on a personal examination of five cases. The gross morbid anatomy is not characteristic. The vessels are bigger than normal and may be nodular. They appear to be solid cords with little or no lumen. Histologically the lesions are much more striking and characteristic (Plate Ia) but vary a good deal in their distribution. At different levels within one short segment of artery the lesion may vary in its intensity, in the presence or absence of the characteristic giant cells, and in the proportion of the circumference involved. In a suspected case, if the histology is atypical it is wise to cut deeper into the block. The lumen is usually reduced to a minute hole or slit and may be thrombosed, but thrombosis is the exception rather than the rule. It has been stated to be present thirteen times and absent thirty times, and probably represents no more than an incidental complication. It is certainly true that thrombosis plays no essential

part in the development of the lesion—a significant point of differentiation from Bürger's disease. The intima shows gross thickening, thus accounting for the diminished lumen. This is invariably present and has been mentioned in every histological report. The intimal thickening may be either concentric or eccentric, usually depending on whether the whole circumference of the media or only a part of it is affected.

During the active stage of the disease this intimal proliferation is often divisible into two different zones (Plate V). The inner one, which is usually the thicker, consists of a cellular fibrous tissue of loose texture with relatively few inflammatory cells or vessels. In the interstices between the fibroblasts there is a pale-staining background which has been described by Sjövall and Windblad (1944) and Gordon and Thurber (1946) as oedema, and by Brown and Hampson (1944), and by Gilmour (1941) as mucoid. In the cases which I have examined this material has been of mucoid nature (Plate IVa) and apparently identical with the mucoid interstitial tissue described by Schultz (1922) and Ssolowjew (1924) in all arteries, and so constantly seen in excess in medionecrosis of the aorta. It seems probable that it represents a normal product of the connective-tissue cells of arteries.

In the outer part of the intima the picture is usually one of a much more active inflammatory response, but this is not always so; some arteries lack this zone entirely (Plate IIb). There are often young capillaries (Plate V) and nearly always an inflammatory cellular infiltration. The cell response varies from one case to another. Lymphocytes, macrophages, plasma cells, and polymorphonuclears may be present in variable proportions, and some giant cells may be seen. Eosinophils are rarely present and are practically never a marked feature—a point of distinction from polyarteritis nodosa. The media usually shows the maximum damage and is often virtually destroyed. Frank coagulative necrosis is frequently present (Plate V) and has been specifically mentioned in twenty-one reports. Cellular infiltration is always present and is rather variable. Lymphocytes and macrophages always appear to be present (Plate IIb and III), and there are usually some polymorphonuclears but they are rarely the dominant cell. Eosinophils are occasionally mentioned (Bowers; Hoyt and others; Kilbourne and Wolff) but never as a dominant cell.

The characteristic giant cells (Plate IIb and III) are mentioned as present in thirty-eight cases and can be inferred to have been present in most of the others. They were apparently not present in the

cases of Kilbourne and Wolff, Gilmour (Case 4), and Sjövall and Windblad (Case 2). I have seen a case in which no giant cell was present in the first few sections of a biopsy specimen. but plenty were present deeper in the block, and it seems probable that all or nearly all cases will show giant cells if the specimen is cut at different levels. These giant cells are of foreign-body type and of irregular shape, sometimes rounded, sometimes elongated. They vary in size from cells of 10μ with two or three nuclei, up to masses of 100μ with very many nuclei. The latter may be distributed in any way, usually being quite haphazard, but sometimes arranged round the periphery exactly like the giant cells of a tubercle. The nuclei resemble those of the neighbouring macrophages, and the cytoplasm has an eosinophilic ground-glass appearance. Some of these giant cells lie in relation to fragments of elastic tissue (Plate IVb) and presumably represent an attempt at its removal, but others occur in the thickened intima in situations away from demonstrable elastica. The internal elastic lamina usually shows severe destruction (Plate IIb). This is recorded in twenty-eight cases and can be inferred from some of the other reports. It is generally fragmented into short lengths, and these may disappear. Sometimes the fragments assume odd shapes. In some cases there is a later overgrowth of new elastica (Cooke and others). These medial lesions are generally rather diffuse, involving large segments or the whole circumference, but in some cases they occur in a curiously focal form. This was so in Barnard's case, where they resembled tubercles, and is beautifully depicted in Fig. 4 of the paper by Cooke and others (1946).

The adventitia is involved to a variable extent (Plate IIa). The adventitial lesions were stressed by Horton and others in their original papers, but subsequent observers appear to have been more impressed by the medial changes. Cooke and others suggest that the lesion spreads along the vessels by the vasa vasorum in the adventitia. In the majority of cases there is a cellular infiltration of the adventitia, contiguous with and similar to that of the media but lacking giant cells. This is usually associated with an overgrowth of adventitia fibrous tissue. The vasa vasorum may be cuffed by inflammatory cells; this has been stressed by Gordon and Thurber and by Kilbourne and Wolff. In some cases nerves are visible embedded in the fibrosed adventitia (Lucien and others, 1939; Cohen and Harrison, 1948). Such involvement of nerves may account for the pain in the temporal regions and for its relief. when a biopsy is performed.

Giant-cell arteritis in larger arteries.—In the cases which have come to autopsy the morphology of the lesions in the larger arteries has been essentially similar to that in the temporal arteries. Furthermore, the descriptions in the clinically uncertain cases of Barnard, Gilmour, and Sproul and Hawthorne tally exactly with those of the clinically proved cases of Cooke and others and of Sproul.

Macroscopically the vessels (aorta, carotid, etc.) may not show any obvious lesion or they may show a smear of fibrin clot overlying the lesion. Microscopically the damage affects mainly the media. The intima may show some recent fibroblastic proliferation, but this is slight and may be absent. Cellular infiltration of the intima is the exception rather than the rule. In the media the striking lesion is a cellular infiltration in which lymphocytes and plasma cells dominate, though a few polymorphonuclears may be seen. Giant cells are always present (Gilmour's Case 4 is an exception) and are of the same type as those in the temporal arteries. They may be found in association with fragments of elastica. Necrosis is usually only a cellular one, with disappearance of muscle in the affected areas, but occasionally foci of dead tissue may be seen. Some destruction of elastica is nearly always present, but it is slight and affects only few fibres. There may be increased vascularity of the media, but this is not striking. In the adventitia, changes are slight or absent. There may be some fibrosis, and there may be some cellular infiltration similar to that of the media.

In arteries intermediate between the common carotid and the temporal the lesions are also intermediate, and there is a greater tendency to thickening of the intima and to thrombosis.

From a study of these cases there seems to be no reasonable doubt that the giant-cell arteritis observed in the larger arteries is the same as that in the temporal arteries and that the disease is widespread.

Aetiology

Nothing is known of the aetiology of giant-cell arteritis. There are, however, certain points which appear significant. It affects white races only; at least no cases have been recorded in coloured races. The early cases (Horton and others) were mostly in farmers and country dwellers, but this has not been the rule in subsequent cases. On the whole, middle-class and well-to-do patients seem to have been rather more frequently affected, but no occupation has been unduly represented. There is no evidence of a familial incidence. No organisms have been recovered with any regularity from

(a)

(b)

PLATE I.

(a)

(b)
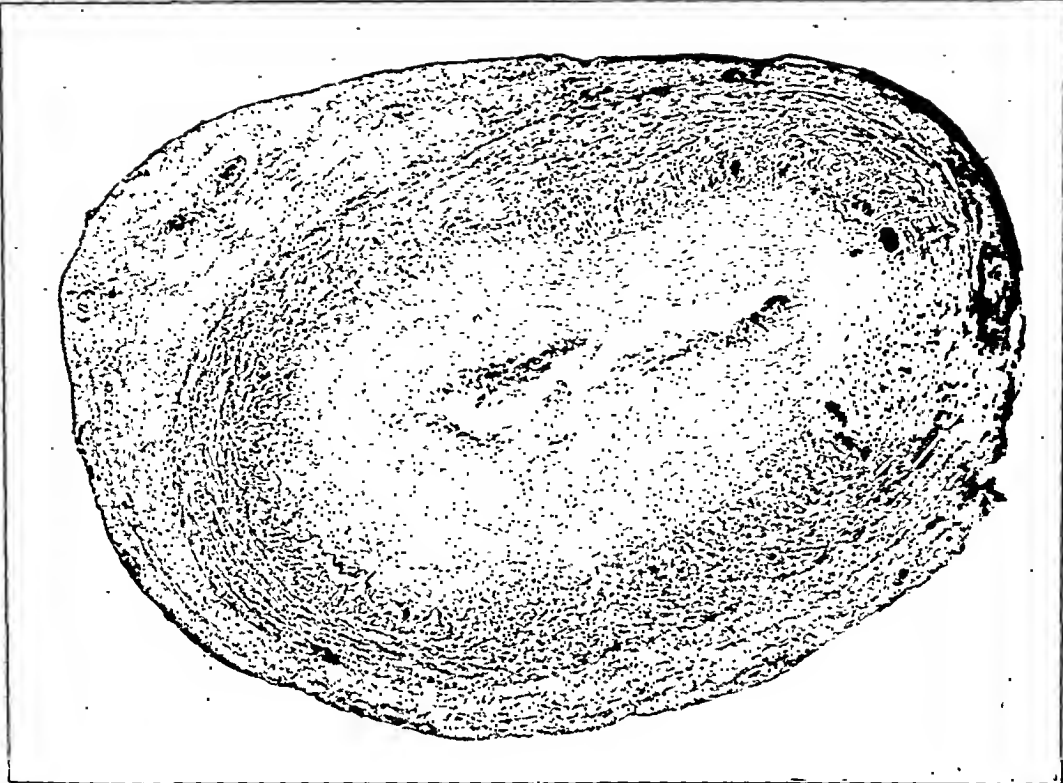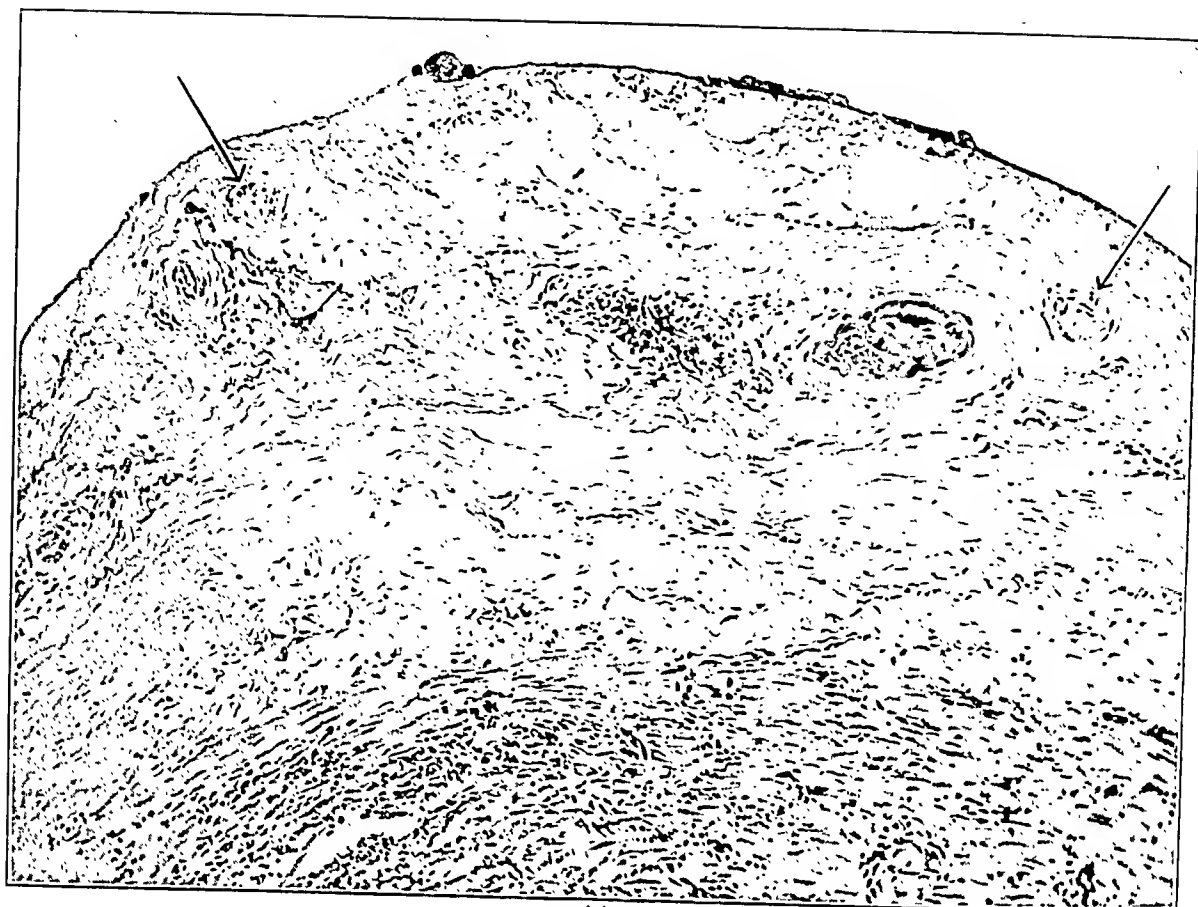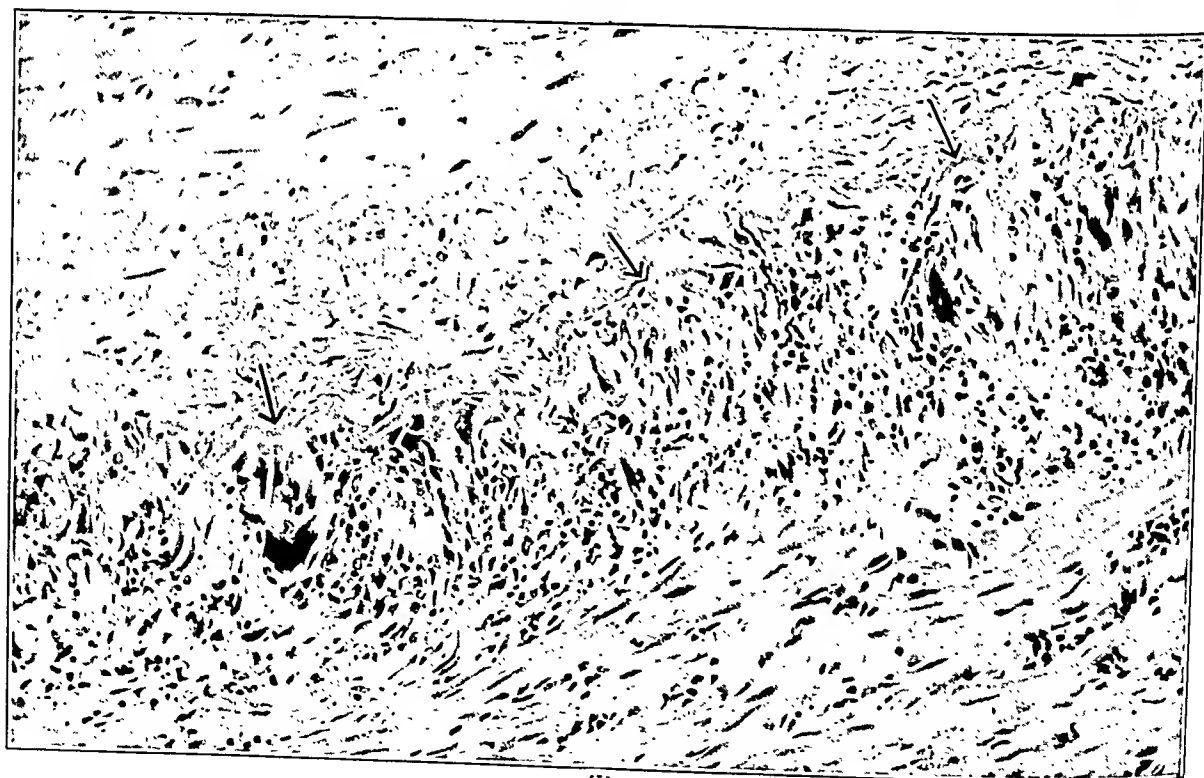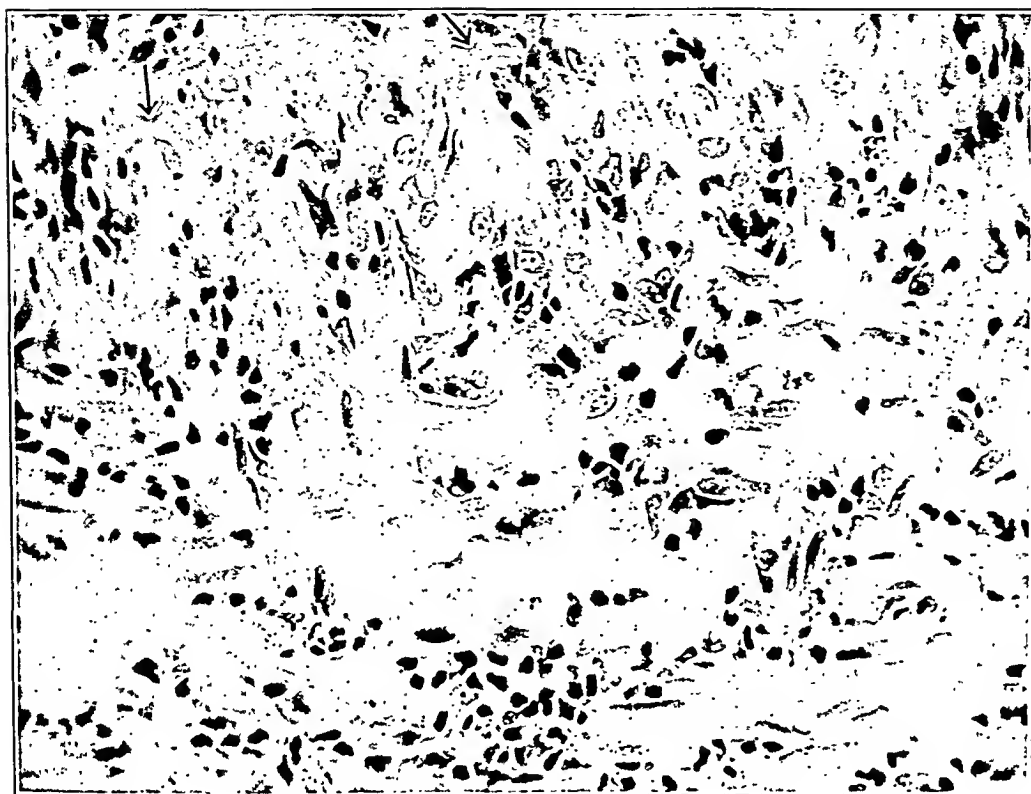
PLATE II.

PLATE III.

PLATE I.—(*a*). Section of temporal artery. There is a little mural thrombus adhering to the intima which is greatly thickened. The media is still visible but heavily infiltrated, and it contains many giant cells. The adventitia is fibrosed. (Haematoxylin and eosin, × 40.) (*b*). Same case as (*a*). Showing the fragmentation of the internal elastic lamina and the formation of new elastic fibres in the deeper part of the intima. (Weigert's elastic and neutral red, × 80.)

PLATE II.—(*a*). Same case as Plate I. Showing adventitia and outer part of media. The adventitia is fibrosed and shows a little cellular infiltration. Note two small nerves embedded in fibrous tissue. (Haematoxylin and eosin, × 120.) (*b*). Same case as Plate I. The intima (upper left) consists of cellular fibrous tissue. The internal elastic lamina (arrows) is fragmented. The media is heavily infiltrated with inflammatory cells and there are numerous giant cells near the elastic lamina. Note that the innermost layer of the media is more severely affected than the rest. (Haematoxylin and eosin, × 190.)

PLATE III.—Same case as Plates I and II. Innermost layer of media showing infiltration with lymphocytes and macrophages and the formation of giant cells, apparently from macrophages. The arrows indicate fragments of the internal elastic lamina. (Haematoxylin and eosin, × 430.)

(a)

(b)
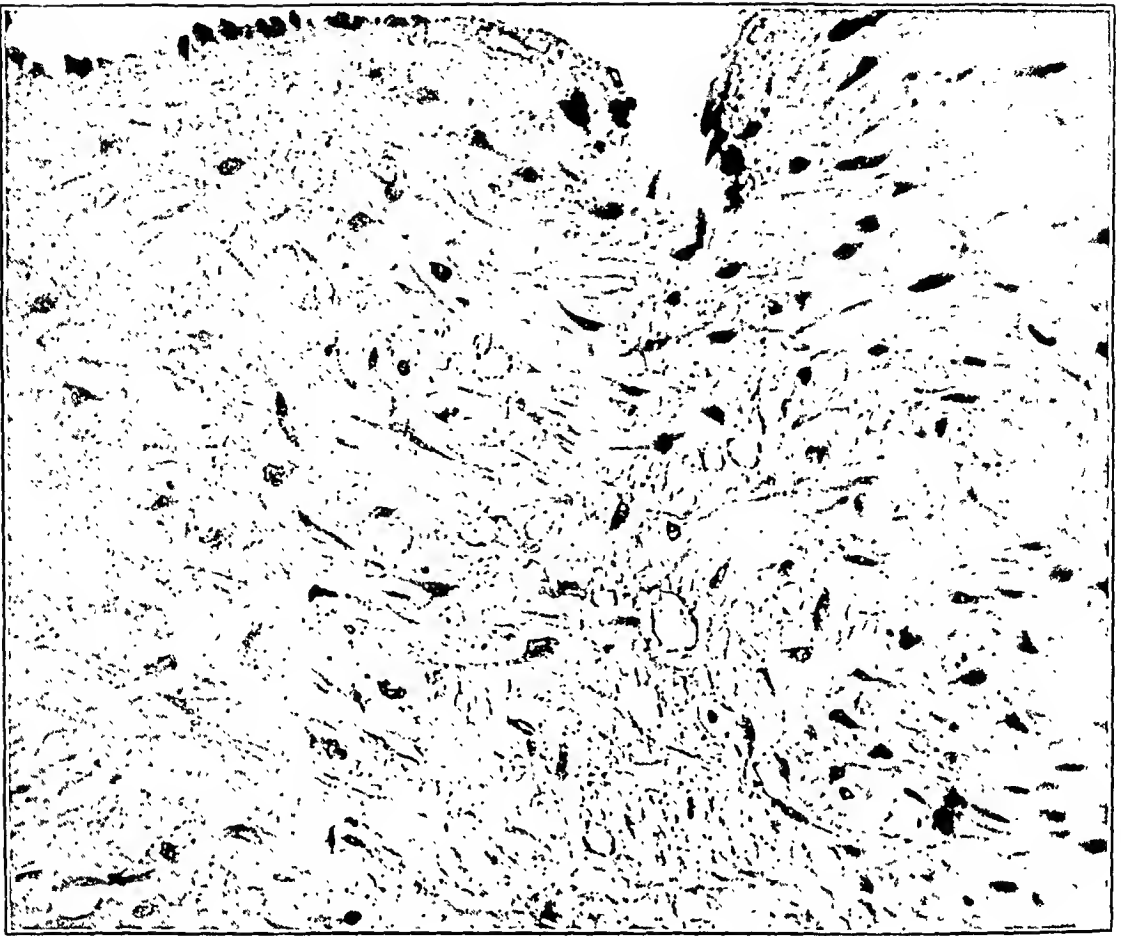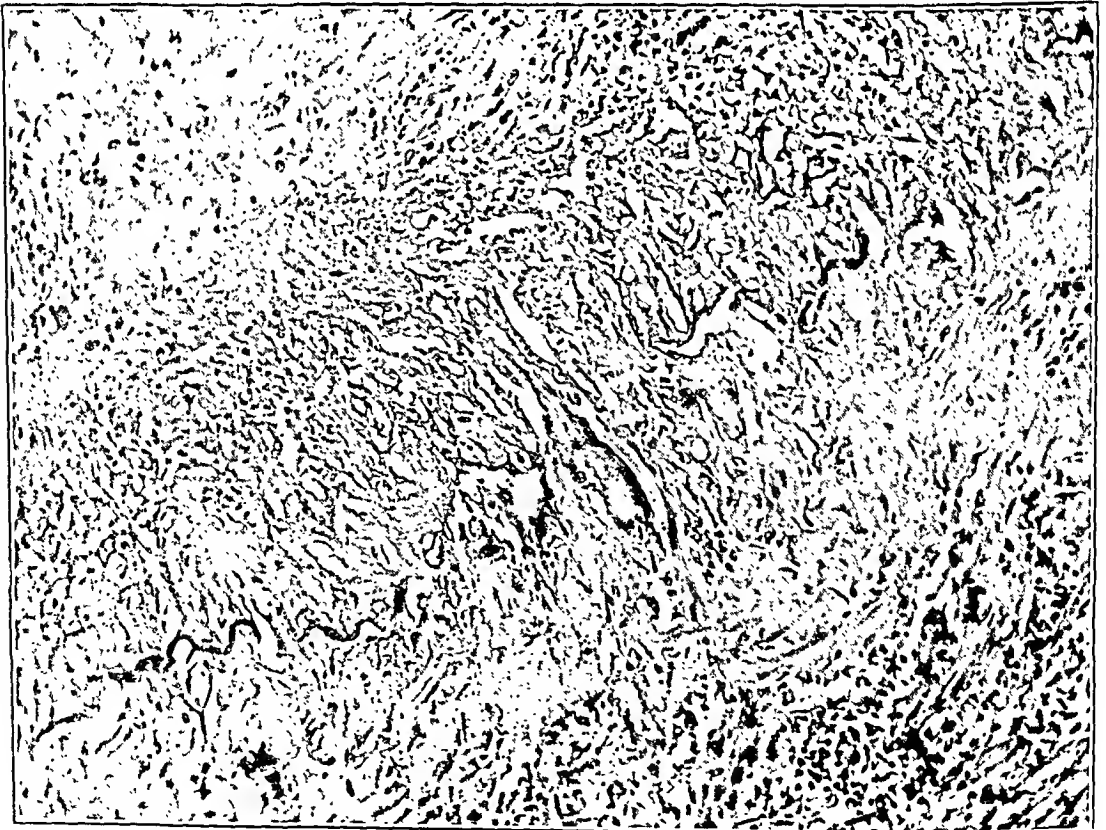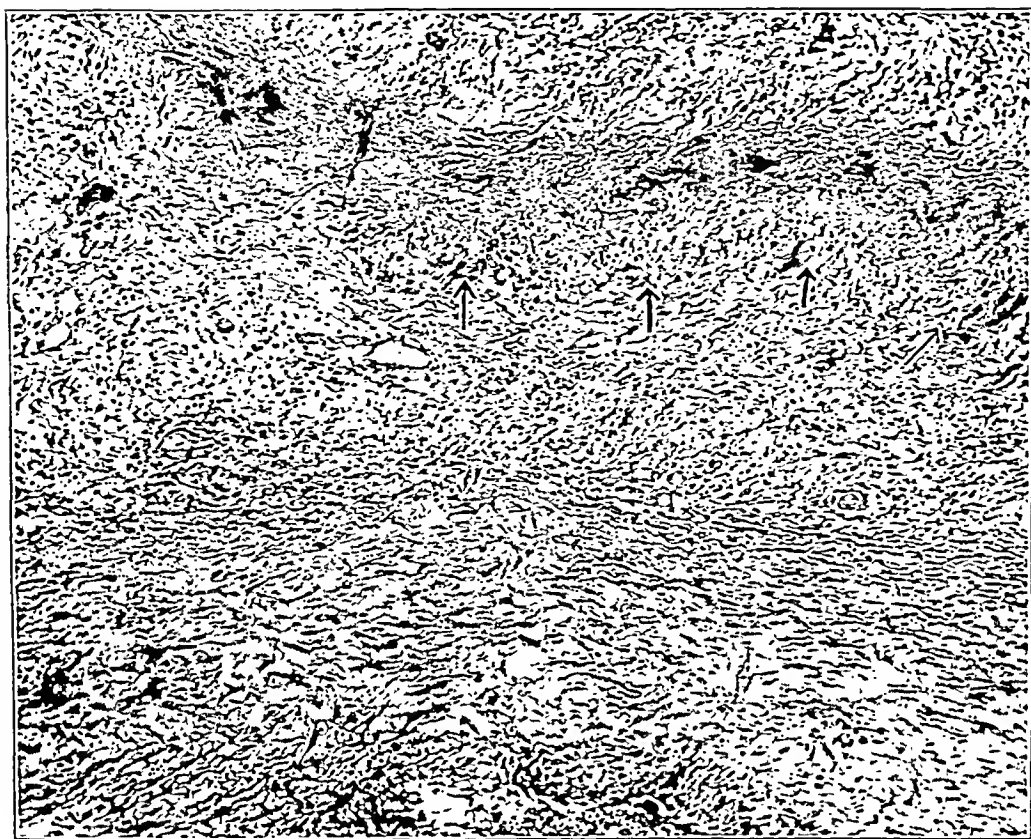
PLATE IV.

PLATE V.

PLATE IV.—(a). Temporal artery. Second case. Innermost layer of thickened intima showing proliferating fibroblasts lying in a background of mucoid connective tissue. (Haematoxylin and eosin, ⨉ 460.)
(b). Same case as (a). Junction of intima and media showing giant cells lying against fragments of the internal elastic lamina on its medial side. Note the cellular infiltration of both media and intima. (Verhoeff and Van Gieson, ⨉ 190.)

PLATE V.—Temporal artery. Third case. The lumen is just visible at the bottom of the section. The inner part of the intima consists of active mucoid fibroblastic connective tissue. The outer part of the intima consists of vascular granulation tissue. The remnants of the internal elastic lamina are shown by arrows. The media shows a band of necrosis. (Haematoxylin and eosin, 120.)

excised arteries. Horton and others grew a type of actinomyces from one of their early cases but decided that this was a contaminant, and certainly no one has since grown a similar organism. MacDonald and Moser grew a staphylococcus which they regarded as a contaminant. Other workers (Andersen; Lucien and others; Scott and Maxwell) have failed to grow any organisms from excised arteries. All attempts to grow organisms from blood culture have been unsuccessful (Andersen; Chasnoff and Vorzimer; Schaefer and Sanders). Finally, many workers have searched for organisms in stained sections, all without success. Yet in spite of these completely negative results, there is indirect evidence which suggests that the disease may have an infective basis; it certainly seems to have an association with infection. Gilmour's four cases all began with an influenza-like onset, and Schmidt's dated from an attack of influenza. Dick and Freeman's first case followed an attack of pneumonia, and Paviot and others' case followed the extraction of a septic molar. MacDonald and Moser's case had a dental-root abscess, and Brown and Hampson's was associated with dental sepsis. Dick and Freeman's second case and Hoyt and others' case had faucial sepsis. All these may have been coincidental, but the fact that one of Horton and others' cases recovered after the extraction of septic teeth, and that Profant's case showed a clinical flare-up after the extraction of a septic tooth, strongly suggests that the arteritis and the sepsis were related. Paviot and others' case had further interest in that this subject also had active rheumatic carditis at the time, and both this and the arteritis responded dramatically to salicylates—a form of therapy which no one else records having tried except for the use of aspirin as an analgesic. Lastly, both of Sjövall and Windblad's cases developed crippling arthritis, whilst Andersen's was left with "rheumatoid pain" in his lower limb.

Apart from its apparent associations with infection there are features in the disease itself which suggest infection. The fever, the leucocytosis, the enlarged cervical glands, and the high sedimentation rate, together with the general illness of the patient, all suggest some sort of infective process. Naturally, the failure to find any organism in a disease of an apparently infective type suggests an allergic response, but Dantes (1946) rejects this hypothesis on account of the lack of eosinophils in the lesions and of other signs of allergy in the patients. So far apparently no attempt has been made to isolate a virus. Thus, at present the data available suggest that giant-cell arteritis is possibly of infective origin, but there is no clue as to the type of infection or the mode whereby infection produces the changes.

Comparison with Other Diseases

A number of workers (Jennings; Gordon and Thurber; Kilbourne and Wolff; Hoyt and others; Andersen) have compared giant-cell arteritis with other arterial diseases, particularly thromboangiitis obliterans and polyarteritis nodosa. Only with the latter does it bear any close comparison, and the question arises whether giant-cell arteritis has sufficient characteristics of its own to justify regarding it as a separate entity and, if so, how closely it is allied to polyarteritis nodosa. If one analyses the clinical picture and the morphology of the arteries, as some workers have done, then there are many points in common and no feature which is absolutely characteristic of either disease. Nevertheless if giant-cell arteritis—either as a clinical entity or as a histological picture—is viewed as a whole, then there is no question of its not being a real recognizable entity.

The clinical differences between giant-cell arteritis and polyarteritis nodosa depend on two basic factors: age, and the arterial territory involved. Polyarteritis is a disease of young adults and affects the small muscular arteries in the viscera: giant-cell arteritis is a disease of the elderly and affects the elastic and larger muscular arteries proximal to the point where they break up to enter the viscera. The histological differences depend largely on the acuteness of the process. Polyarteritis is often acute, whilst giant-cell arteritis is subacute. The signs of an active infection—the fever, illness, leucocytosis, anaemia, etc.—are common to both. The clinical pictures depend for their differentiation on the territory involved. The differentiating signs of giant-cell arteritis—the headache, palpable temporal arteries, eye involvement, and nervous signs—depend on the involvement of the larger cranial vessels. Similarly the signs of polyarteritis—the renal lesions, cardiac signs, abdominal signs—are the result of visceral ischaemia from involvement of small visceral arteries.

In a similar way the histology of giant-cell arteritis (the lymphocytes and plasma cells, the giant-cells, the fibroblastic reaction) are all those of a subacute disease, whilst the histology of polyarteritis nodosa (the polymorphonuclears and eosinophils, the fibrinoid necrosis, the aneurysms) are all those of an acute process.

There is one final point of difference: polyarteritis is probably an allergic response (Rich and Gregory, 1943; McKeown, 1947); whilst there is no

evidence, either clinical, morphological, or experimental, to suggest that giant-cell arteritis is of this character.

Summary

1. Giant-cell arteritis is a disease of the elderly of either sex.

2. It runs a course of about six months and is characterized by malaise, fever, severe headaches and prominent, thick, painful temporal arteries; it is usually accompanied by leucocytosis, anaemia, and raised sedimentation rate.

3. There is no specific treatment, but the disease tends to subside slowly and complete recovery is the rule.

4. It may be complicated by blindness or cerebral disturbances from involvement of appropriate arteries.

5. In some cases the disease has proved fatal, and it has been shown to involve many arteries of the elastic and large muscular group, from the aorta down.

6. In most cases a number of cranial arteries are involved.

7. The arterial lesions are characterized by lymphocyte and plasma-cell infiltration of the media with frequent giant cells. The elastica is usually broken up, and the intima undergoes fibroblastic proliferation. There is usually some fibrosis and cellular infiltration of the adventitia. The disease appears to be infective but its aetiology is unknown.

8. The relationship of the disease to polyarteritis nodosa is discussed.

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TEMPORAL ARTERITIS: A REPORT OF THREE CASES

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This disease, which has also been described as cranial arteritis and giant-cell arteritis, was first established as a clinical and pathological entity by Horton and others (1934), though there is evidence that single cases were described before this (Andersen, 1947). Since 1934, over seventy cases have been reported in the literature from U.S.A., Great Britain, France, and Scandinavia. These have been reviewed and analysed elsewhere in this issue (Harrison, 1948). The disease runs a characteristic clinical course, and the affected vessels have a characteristic morphology. Although the earlier recorded cases ran a completely benign course, those subsequently reported have shown that involvement of other vessels may cause blindness, cerebral damage, and even death.

The three cases reported here exemplify the characteristic clinical course and morphology of the disease, and also illustrate some of its complications.

Case Reports

Case 1.—A coach builder, aged 72, from a country district of North Wales was admitted to hospital with a three months' history of shooting pains in both sides of his head. The pain was at first intermittent and located behind the left ear, but later it became almost continuous and spread to the front and back of the head. Six weeks before admission the pain moved to the right temple and came in bursts of great severity alternating with a continuous dull ache. Bending down made the pain worse but coughing and eating did not. During the two weeks before admission the pains had eased a little. On examination he was a well-nourished man with normal temperature and pulse ; his face showed leucoderma. Both superficial temporal arteries were visibly prominent but no longer tender. There was no pulsation in ei her. Physical examination (blood pressure 148/80 mm. Hg.), blood count, electrocardiogram, and radiograph of the chest all gave negative results.

A segment of the right superficial temporal artery was excised, and this appeared to relieve the pain. After nineteen days in hospital, that is, about 15 weeks after the onset of symptoms, the patient was discharged much improved.

Histology (Plate VIa and b).—The lumen was reduced to a small slit by intimal proliferation, but there was no thrombosis. The intimal thickening was composed of a cellular connective tissue containing fibroblast nuclei but very few inflammatory cells. The media was intact around approximately two-thirds of the circumference and was infiltrated by a few inflammatory cells. In the remaining third it was totally replaced by a cellular infiltration of macrophages, lymphocytes, and polymorphonuclears, though there was no remaining necrotic tissue. The internal elastic lamina was fragmented, and there were a few giant cells in the vicinity of the broken elastic fibres. The adventitia was fibrosed, forming a thickened collagenous coat, and several nerves were embedded in it. It did not, however, show any appreciable cellular reaction.

Case 2.—A farmer aged 65 years was admitted complaining of headaches. Six weeks before admission he felt ill and took to bed and thought that he was febrile. Soon after this he developed headaches all over his head but worse on the left side. This was aggravated by coughing and was severe enough to interfere with sleep. He had lost about 1 stone in weight since the onset of his illness. On examination he was found to be well preserved, but he showed signs of recent loss of weight. He had some pyrexia (98 to 101° F.) on admission, but this settled to normal in a few days. The temporal arteries were tortuous, thick, and tender, but pulsating. Blood pressure was 120/80 mm. Hg. Abduction of the right shoulder was limited and the right deltoid wasted. Physical examination was otherwise negative. Blood examination revealed Hb 75 per cent, red blood cells 4,400,000, white cells 12,800 per c.mm. of blood, with 73 per cent polymorphs. The erythrocyte sedimentation rate was 10 mm. in 1 hour (Wintrobe-corrected), the

(a)

(b)

(c)

PLATE VI.—(a). Case 1. Transverse section of temporal artery showing extreme intimal proliferation. In the segment on the left the media is largely destroyed and the elastica is interrupted. The adventitia is fibrosed. (Verhoeff and Van Gieson, · 50.) (b). Case 1. High-power magnification of part of (a), showing the cellular infiltration spreading through media. A giant cell is shown near the left side. (Haematoxylin and eosin, · 140.) (c). Case 2. Slightly oblique section. The intima is enormously thickened and the inner fibrous and outer granulomatous layers are shown. The media is well seen near the lower border but is necrotic near the upper border. The adventitia is fibrosed, and a small nerve is visible at the extreme lower edge. (Haematoxylin and eosin, 38.)

PLATE VII.—(a). Case 2. Higher magnification of part of Plate VIc, showing granulomatous replacement of media spreading into intima (lower left). Two giant cells are seen near the top left corner. (Haematoxylin and eosin, × 190.) (b). Case 3. Transverse section just beyond a bifurcation. The greatly thickened intima is composed of loose connective tissue. The media is infiltrated by cells, and the adventitia is fibrosed. (Verhoeff and Van Gieson, × 45.) (c). Case 3. Higher magnification of part of (b). The intima lies to the left and the media to the right, separated by a wavy black elastic lamina. Note the numerous giant cells in the media. (Verhoeff and Van Gieson, × 120.)

(a)

(b)

(c)

Wassermann reaction negative, and the cerebrospinal fluid normal.

A week after admission a segment of the left temporal artery was excised and this was followed by relief of the headache. A fortnight after admission, eight weeks after the onset of the illness, the patient was discharged free from pain. Six months later he died suddenly from coronary occlusion: no autopsy was performed.

Histology (Plates V, VIc, and VIIa).—The lumen was reduced to a slit 0.1×0.5 mm. but was free from thrombus. The intima was greatly thickened and consisted of two layers. The inner was composed of mucoid connective tissue with fairly numerous fibroblast nuclei but very few inflammatory cells; the outer consisted of granulation tissue with numerous capillaries and fibroblasts and heavily infiltrated with polymorphonuclears and macrophages. The media around approximately half the circumference was necrotic; and the rest, though containing muscle fibres, was heavily infiltrated by polymorphonuclears, lymphocytes, and macrophages. The internal elastic lamina was disrupted, and there was a number of giant cells. The adventitia was fibrosed but contained few inflammatory cells. Several small nerves were embedded in the fibrous tissue. A small branch of the temporal artery included in the biopsy showed exactly the same lesion as the parent vessel. A biopsy of calf muscle did not show any vascular lesion.

Case 3.—A farmer aged 69, and a neighbour of Case 2, was admitted complaining of headache and blindness in one eye. Five months before admission he developed severe pain in the right side of his head; a fortnight later he suddenly developed diplopia for five minutes and then lost the sight in the left eye. A fortnight later the pain on the right cleared up but soon returned on the left side, and he noticed a " swollen vein " in his left temple, which he said was not tender. Two months after the onset of the illness the pain returned to the right side and persisted there till admission. It was severe enough to interfere with sleep.

On examination he was seen to be a wasted elderly man, apyrexial, but with a pulse rate of 100 to 120 per minute. The left superficial temporal artery was thick and tortuous but not tender. The right temporal artery was not palpable. The left eye was blind except for appreciation of light and dark; the disc was pale and there were signs of thrombosis of the central retinal artery. The blood pressure was 175 80 mm. Hg, and there were signs of generalized arteriosclerosis with some myocardial ischaemia. The sedimentation rate was 32 mm. in one hour (Wintrobe-corrected). The blood count showed Hb 90 per cent. red blood cells 4,300,000 and white cells 6,200 per c.mm. The cerebrospinal fluid was normal.

A segment of the left temporal artery was excised and this was followed by relief of his pain. He was discharged free from symptoms a fortnight after admission: five and a half months after the onset of his illness.

Histology (Plates IVa and b, VIIb and c).—The specimen consisted of a Y-shaped segment and was cut to show transverse sections of the two limbs. These were exactly similar. The lumen was patent but reduced to a narrow slit. The intima was greatly thickened and consisted of mucoid connective tissue with frequent fibroblast nuclei but very few inflammatory cells. The media was free from any gross necrosis but was largely destroyed by a granulomatous mass consisting of young capillaries, polymorphonuclears, lymphocytes, and a few macrophages. Giant cells were numerous. This acute inflammatory reaction spread into the immediately adjacent intima and adventitia, but only for very short distances. The internal elastic lamina was broken up into a line of short fragments. Only the inner part of the adventitia was included in the biopsy, but this showed some fibrous thickening. No nerves were included.

Comment

The present cases were all in males, though in the recorded cases there have been slightly more women than men (Andersen, 1947; Cooke and others, 1946). The ages of the present cases, 72, 65, 69, are in accordance with the usual findings, most recorded cases being over 60.

It is of interest that our three cases were country dwellers, and two were neighbouring farmers admitted within three months of each other. Horton and others (1934) drew attention to the fact that their early cases were farmers or country dwellers, though in most of the subsequent publications the patients have either been town dwellers or this information has not been given.

The duration of the disease in our cases was 15, 8, and 23 weeks, which is shorter than the average (about six months) of previously recorded cases. But it is within the range of duration of such cases, which has varied from seven or eight weeks (Hoyt and others, 1941; Sproul, 1942) up to two years' (Cooke and others, 1946).

In this series Case 1 recovered completely. Case 3 also recovered but was left with blindness in his left eye. Case 2 died from myocardial infarction, six months after discharge. An autopsy was not performed, and we do not know whether death was due to arteritis affecting the coronary arteries or to coincidental atheroma. Of the published cases, a number have died within some months of discharge, mostly from myocardial ischaemia or cerebral vascular disease. (Chasnoff and Vorzimer, 1944; Curtis, 1946; Kilbourne and Wolff, 1946). Other cases (Cooke and others, 1946) have died during the course of the disease from cerebral ischaemia due to arteritis of the cranial arteries and the diagnosis confirmed at autopsy.

In the first of the three cases here recorded the lesion appeared to be limited to the temporal arteries; in Case 2 the right circumflex artery was involved, and in Case 3 there was blindness in the left eye due to thrombosis of the central retinal artery. In the early reports it was suggested that the disease was a localized one, but Jennings (1938) described blindness as a complication presumably due to involvement of the retinal vessels, and similar cases have since been reported by Dick and Freeman (1940), Scott and Maxwell (1941), Cooke and others (1946), Shannon and Solomon (1945), Robertson (1947), and Curtis (1946). The cases that have come to autopsy have shown involvement of other vessels.

That the disease is not a localized process is suggested by the frequent occurrence of pyrexia and pains in body and limbs, and a degree of systemic illness out of proportion to the physical findings. Case 2 of the present series was pyrexial and was sufficiently ill to take to bed. The outstanding symptom in our three cases, and in all the recorded cases, has been pain in the head. This is of great severity and is usually resistant to analgesics. Many forms of therapy have been tried but none has been constantly successful. Removal of a segment of the affected temporal artery for biopsy has quite frequently been followed by relief of pain. This was noted by Horton and Magath (1937) and has since been confirmed by numerous other workers, and from the literature it appears to be the most constantly successful form of therapy. It was certainly of value in relieving symptoms in the present three cases. It has been suggested that this is due to interruption of the accompanying nerves, and

Lucien and others (1939) described such nerves embedded in the fibrosed adventitia. We have also noted nerve fibres embedded in the fibrosed adventitia of our first and second cases.

Nothing is known of the aetiology of the disease and, though many of the clinical features suggest an infective cause, so far all attempts to isolate an organism have been fruitless. Nevertheless the disease presents a uniform pattern which differentiates it from polyarteritis nodosa or Bürger's disease. It has a different anatomical and age incidence and a better prognosis. The histology of the lesions is also different though in some ways less characteristic. Almost any of the individual histological features of temporal arteritis may be found in either polyarteritis or Bürger's disease, yet the whole picture is sufficiently distinctive to be recognizably different (Gilmour, 1941 ; Cooke and others, 1946).

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THE GASTRIC NEUTRAL-RED EXCRETION TEST

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Introduction

The stomach's acid secretion is commonly used as a measure of gastric function, and various standardized tests have been devised. Owing, however, to the wide range of acid secretion in normal people, to the inconstant results obtained by repeating a test on many individuals, to the frequent occurrence of results well within the normal range in patients suffering from gastritis, gastric carcinoma, or peptic ulcer, and to the fact that from 10 to 20 per cent of normal people may have spontaneous temporary or permanent achlorhydria (Schiff, 1938 ; Palmer and others. 1940), estimation of the acid secretion of the stomach after a popularity of many years is now falling into disfavour as a diagnostic test.

In the search for a more reliable test, the excretory function of the gastric mucosa to various substances including dyes has been studied: one of these dyes is neutral red.

When a solution of neutral red is injected into a vein of a normal individual. the dye is *excreted* within a few minutes into the gastric juice, staining it pink: the concentration increases for a period and thereafter diminishes. During the next twenty-four to forty-eight hours the dye is excreted in the urine and colours the faeces.

In 1923 Glaessner and Wittgenstein introduced the excretion of neutral red by the stomach as a test of gastric function. but the normal variations were not worked out, nor was the relationship of dye *excretion* to acid *secretion* fully understood by these or subsequent workers (Davidson and others. 1925; Zibalis, 1934; Winklestein. 1942; Lourja and Mirkin, 1925). Twenty years later the excretion of neutral red by the stomach was reintroduced by Gillman (1943, 1944) as a test of gastric function. He used a standard technique whereby the rate of

dye excretion and the rate and intensity of concentration of the dye in the gastric juice could be measured. He worked out the normal variations in healthy young European adults in Johannesburg, and showed that normal results were repeatable within narrow limits. By studying the results obtained on three hundred cases, mostly African natives with disturbed gastric function, he claimed to have established criteria of abnormality which could be of diagnostic and prognostic aid.

The intention of the present study was to check the range of normality reported by Gillman : to study the anatomy and physiology of neutral-red excretion ; to compare the acid secretion of the stomach with its power to excrete dye. and to determine if the test could be of use in this country in the diagnosis of various gastric and duodenal disorders.

Methods

Technique of the neutral-red excretion (N.R.E.) test.—The Gillman method was adhered to with only minor modifications. A Ryle or Rehfuss tube was passed through the mouth into the stomach of the patient. who had fasted overnight. After the resting juice had been drawn off. the stomach was washed out with 100 ml. or more of warm water by means of a 20-ml. syringe until the aspirate became clear. When little or no mucus appeared in the washing. the stomach was emptied and was ready for the test. In a few cases. especially in carcinoma of the stomach. it was found difficult or impossible to obtain a clear and clean aspirate: these were cases with great excess of mucus in the juice. often together with altered blood.

Without delay. 5 ml. of a 1 per cent sterile neutral-red aqueous solution was injected intravenously as rapidly as possible. and a stop-watch was started. Any remaining stomach contents were removed as soon as the injection was completed. Subsequent aspirations were carried out at 1-minute intervals

until the dye first appeared in the juice. After that, aspiration was repeated every 2 minutes for the next 15 to 20 minutes, and later at 3- to 5-minute intervals. The period of the test was at least 30 minutes, and usually ten to fifteen aspirates were made. The entire volume of gastric contents obtainable was removed at each aspirate and placed in a test-tube of 5/8 in. diameter upon which was marked the number of minutes from the time of the dye injection. The complete removal of the gastric contents before and immediately after injection and at subsequent aspirations was considered important : otherwise an unknown dilution factor would occur. Patients were instructed to expectorate and not to swallow saliva excreted during the test.

Difficulty in aspiration sometimes occurred : if the tube was blocked by a plug of mucus, injection of 20 ml. or more of air down the tube often cleared it : sometimes aspiration was facilitated by the patient bending forward.

The concentration of the dye in each sample of gastric juice was estimated by direct vision in a comparator box using standard dilutions of the dye contained in identical test-tubes. The standard tube was backed with a tube containing uncoloured juice, and the unknown with a tube of water. With a little practice the dilutions were easy to read.

The standards were kept in well-stoppered test-tubes ready for use. Deterioration in colour was not appreciable for three or four months. The dye was made up in decinormal hydrochloric acid and the following dilutions used, viz., 1/300,000, 1/200,000, 1/100,000, 1/75,000, 1/50,000, 1/30,000, and 1/20,000. The actual concentration of the dye was not usually recorded but the arbitrary scale of Trace, 1, 2, 3, 4, 5, and 6 used by Gillman was adopted (Trace = 1/300,000, 1 = 1/200,000, etc.). Graphic representation of each result was made by plotting dye concentration of the sample against the time in minutes (see Figure).

Further studies of the fasting juice and subsequent unstained and stained gastric fractions were carried out. The volumes were measured, the presence of mucus, bile, and macroscopic blood were noted, and benzidine tests for occult blood were performed. " Free hydrochloric acid " and " total acid " estimations were performed on the fasting specimen and on four or five samples of juice removed at consecutive 5- to 10-minute intervals. The dye was first removed from the sample by filtration through two or three thicknesses of filter paper, which absorbed the neutral red. Sometimes refiltration was necessary. Titration was carried out on 5 ml. (or if available 10 ml.) of the specimen with N/10 or N/20 sodium hydroxide, using a drop or two of a mixture of Töpfer's reagent and phenolphthalein as indicators. If achlorhydria was present, qualitative tests for pepsin were performed on two or three mixed samples of juice using coagulated egg-white as a substrate.

In some of the cases (*vide infra*) in which excretion of neutral red did not occur or was very poor

within the first 30 minutes, aspirations were continued every 5 minutes for a further 30 to 60 minutes, sometimes after an intramuscular injection of 0.5 mg. of histamine.

Fractional test meals.—These were not performed on the twenty " control " cases, but were carried out on patients exhibiting gastric symptoms, usually a few days before the neutral-red excretion test was performed.

Absence of toxicity.—In the dosage used, neutral red was found to be free from toxic or unpleasant local or general effects. Its excretion in the urine and its colouring of the faeces within the following day or two was physiological. It was found advisable, however, to warn the nursing staff and the patient of this occurrence so as to prevent undue anxiety or alarm.

Observations

Results in N.R.E. tests in patients free from gastro-intestinal symptoms.—N.R.E. tests were performed on twenty in-patients of a military hospital who were free of any gastro-intestinal symptoms or signs. It was difficult to find perfectly healthy patients in hospital, but patient volunteers with various non-gastric disorders were selected. They consisted of six convalescent minor surgical
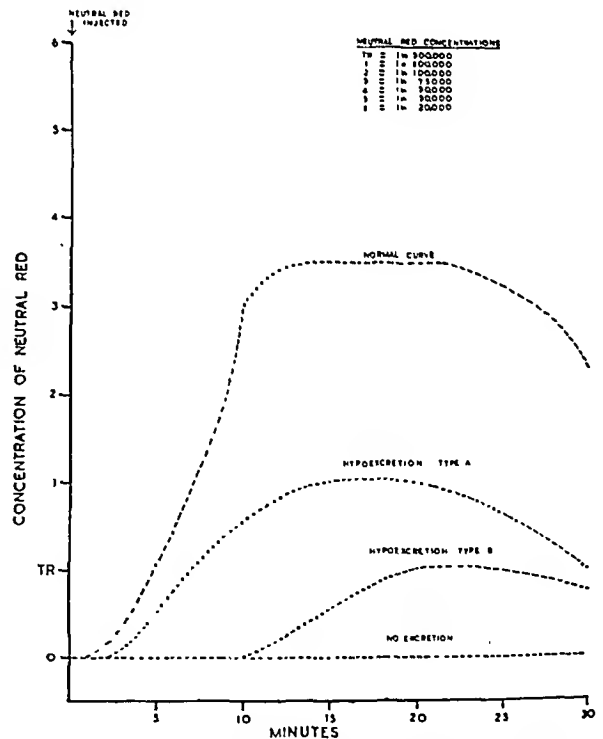
FIGURE.—Normal and abnormal neutral-red excretion curves.
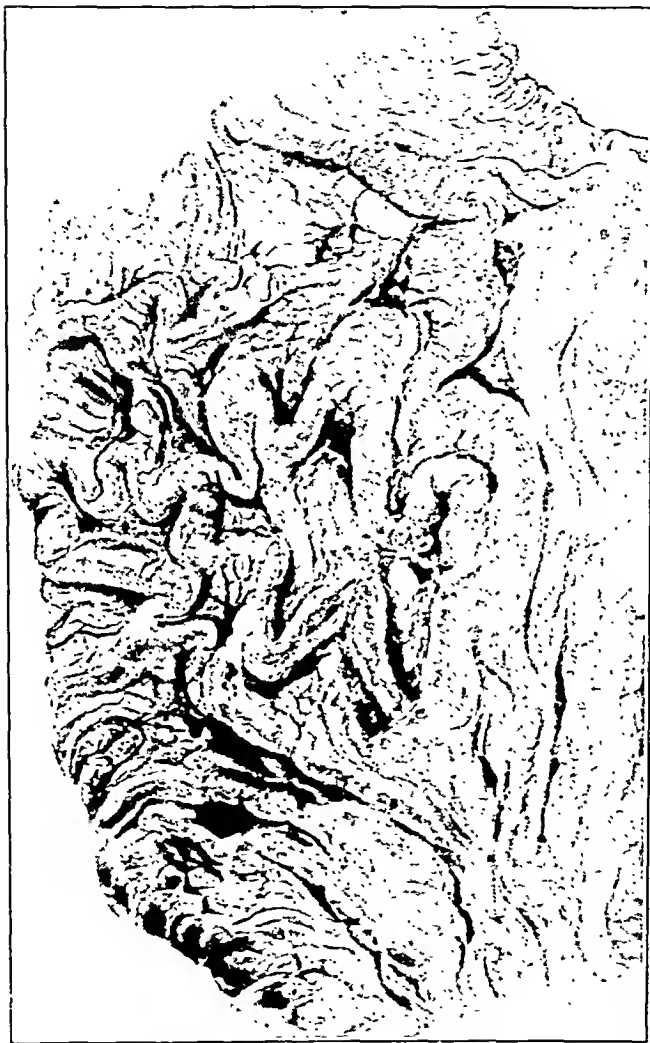
PLATE VIII.—Photograph of a colour drawing showing excretion of neutral red by the mucosa of the upper half of the body of the stomach (black streaks = neutral red).

(Original drawing by Miss D. Davidson.)

and fracture cases, three patients completely recovered from old head injuries, two mildly neurotic patients, and a case each of Bell's palsy, tinea pedis, convalescent atypical pneumonia, endometrosis, convalescent tonsillitis, progressive spastic palsy, congenital cystic disease of the lungs, and convalescent catarrhal jaundice. All these patients were out of bed ; they were fit and well and had good appetites.

Only two of the patients were women ; of the men, four were Germans, one an Italian, another a Pole, and the remainder were English, Scottish, or Irish. Two patients were 35 and 48 years of age respectively, the remainder 18 to 26 years old.

The details and shape of the N.R.E. curves were studied (see figure). The general shape of each curve was similar ; dye tinged the juice a faint pink within a few minutes, and the concentration increased—usually quite rapidly—to reach a maximum which was maintained for a period and which then declined. Particular attention was paid to :

(1) The *excretion time* (E.T.), or the period in minutes between injection of the dye and its first appearance in the aspirate. In all cases this was 5 minutes or less. It is noteworthy that in seven cases dye appeared within 1 minute, in thirteen within 2 minutes, and in seventeen cases within 3 minutes of the injection.

(2) The *concentration time* (C.T.) or the period in minutes between injection of the dye and its earliest maximum concentration in the juice. In fifteen cases this was between 8 and 12 minutes ; in the remainder it was 4, 5, 6, 14, and 19 minutes respectively.

(3) *The maximum degree of concentration found.* (C_M). Using the arbitrary scale of Trace, 1, 2, — 6 (see above), the C_M in 15 cases was between 3 and 5 ; one was 6, three were 2, and one was 1.

TABLE I

RELATIONSHIP OF NEUTRAL-RED EXCRETION TESTS TO THE ACID SECRETION OF THE STOMACH

Group of cases	No. of cases in group	Classification of N.R.E. tests	No. with hyper-acidity	No. with normal acidity	No. with hypo-acidity	No. with achlor-hydria	Total
Non gastric controls	20	Normal N.R.E.	5	14	0	0	19
		Hypo-excretory, Type a	0	1	0	0	1
Carcinoma of stomach	10	Normal N.R.E.	1	2	0	0	3
		Hypo-excretory, Type b	0	0	0	2	2
		N.R. not excreted	0	0	1	4	5
Chronic dyspepsia and chronic gastritis	11	Normal N.R.E.	0	4	1	0	5
		Hypo-excretory, Type b	1	1	0	0	2
		N.R. not excreted	0	1	0	3	4
Peptic ulcer	16	Normal N.R.E.	4	6	0	0	10
		Hypo-excretory, Types a and b	0	4	2	0	6
		N R. not excreted	0	0	0	0	0
All Cases	73	Normal N.R.E.	11	35	2	0	48
		Hypo-excretory	1	6	2	3	12
		N.R. not excreted	0	1	2	10	13
Totals			12	42	6	13	73

Statistical Analysis—Chi-squared testing of all cases was done by grouping of classes owing to the small numbers. In all arrangements used there is a highly significant departure from random distribution ($\chi^2 = 35.06$, 31.3 or 30.09 : $P < 0.0001$). The chief contribution to the Chi-squared comes from the achlorhydric cases.

TABLE II

ANALYSIS OF CASES WITH ABNORMAL N.R.E. TESTS

Case No.	Diagnosis	X-ray findings barium meal, etc.	Laparotomy findings and operation	N.R.E. test			Max. free HCl of gastric juice
				E.T.	C.T.	C_x	
9	Non-gastric control (Bells palsy)	—	—	1	9	1	62%, Normal acidity
21	Gastric carcinoma	Filling defect near lesser curvature	—	Never	Never	Nil	Achylia gastrica "coffee-ground" fasting juice
58	Gastric carcinoma	Irregular filling defect on lesser curvature	Inoperable cancer of lesser curvature	Never	Never	Nil	Achlorhydria
62	Gastric carcinoma	Large ulcer on lesser curvature	Malignant ulcer; secondaries in liver	Never	Never	Nil	8% hypo-acidity
69	Gastric carcinoma	Ulcer on lesser curvature	High, inoperable carcinoma	Trace 9 and 11 mins.	Never	1 at 13 mins. Intermittent excretion	Achlorhydria
71	Gastric carcinoma	Large, pyloric filling defect	Pyloric cancer: partial gastrectomy	Never	Never	Nil	Achlorhydria
72	Gastric carcinoma	Filling defect near cardia	Inoperable carcinoma	Never	Never	Nil	Achlorhydria
59	Carcinoma of transverse colon	Filling defect on greater curvature	Malignant infiltration of greater curvature	20 Intermittent excretion	Never	1	Achlorhydria
2	Chronic dyspepsia	Normal	—	24	Never	Trace	50%, climbing curve in F.T.M. Mucus + + +
4	Alcoholic gastritis (with hepatic cirrhosis)	Normal	—	Never	Never	Nil	Achylia gastrica
10	Chronic gastritis	Normal	—	Never	Never	Nil	Achylia gastrica
28	Chronic gastritis	Normal	—	10 Intermittent excretion	Never	Trace	70% climbing curve in F.T.M. hyperacidity
66	Chronic dyspepsia	Mucosal puckering on lesser curvature, ? gastric ulcer	Stomach, duodenum etc., normal	Never	Never	Nil	42% normal acidity
69	Alcoholic gastritis (with renal colic)	Normal	—	Never	Never	Nil	Achlorhydria
51	Chronic cholecystitis	Normal	—	Never	Never	Nil	Achlorhydria
68	Chronic cholecystitis	Barium meal = normal. Gastroscopy = atrophic gastritis	Cholecystectomy and appendicectomy	Never	Never	Nil	Achlorhydria
8	Gastric ulcer	Ulcer on lesser curvature	—	9	Never	Trace	Trace of free HCl. Pepsin ±. "coffee-ground" fasting juice
25	Gastric ulcer	Ditto	—	3	7	1–2	12% low normal acidity
42	Gastric ulcer	Ditto	—	9	35	1	9% hypo-acidity
20	Duodenal ulcer	Shallow ulcer on medial border of cap	—	2	Never	Trace	62% normal acidity
50	Duodenal ulcer	Ulcer on cap	Partial gastrectomy	5	28	2	60% normal acidity
56	Gastro-jejunal ulcer	(Gastro-enterostomy several years previously)	Vagotomy	Trace 11–20 mins.	25	2	15% low normal acidity
6	Intestinal colic	Barium meal and enema = N.A.D.	—	31	Never	Trace	Achlorhydria Pepsin +
61	Achalasia cardia	Barium meal indefinite	—	Never	Never	Nil	10% hypo-acidity
14	Post-head-injury depression	Normal	—	Never	Never	Nil	Achlorhydria, Pepsin =

E.T. = Excretion Time.   C.T. = Concentration Time. C_x = Maximum Concentration.

Q

The normal curve of N.R. excretion.—Excluding one test, excretion of neutral red by the stomach began within 5 minutes of the injection ; the dye was then concentrated to reach a maximum within 19 minutes, and the maximum concentration reached was between 2 and 6. In the case of Bell's palsy showing a C_M of 1 only, the E.T. was 1 minutes, C.T. 9 minutes (see Table II).

Except for the maximum degree of concentration, these results are in general agreement with those reported by Gillman (1944). He found that normal individuals excreted dye into the stomach within 10 minutes of the injection (usually 4 minutes) and concentrated it to an intensity of 4 to 6 within 20 minutes (average 14 minutes). If Gillman's maximum concentration is to be accepted, then the ten N.R.E. curves with a maximum of -3, 2, or 1 would be considered abnormal. None of these patients had any symptoms or signs of gastro-intestinal disorder, and they formed 50 per cent of the present " control " series.

It was arbitrarily decided to consider a C_M of 1 as abnormally low and to define a normal N.R.E. curve as one in which dye is excreted within 10 minutes and reaches a maximum concentration of 2 to 6 within 20 minutes of intravenous injection of 5 ml. of 1 per cent neutral red.

Neutral-red excretion tests in patients with gastric or intestinal disorders.—Tests were performed in a military hospital and in the Professorial Surgical Unit, Manchester Royal Infirmary, on fifty-three patients with disturbed gastric or intestinal function. The cases fell into eight groups, viz.: gastric carcinoma, peptic ulcer, " duodenitis," gastric neurosis, lienteric diarrhoea, chronic dyspepsia including chronic gastritis and alchoholism, cholecystitis, and a miscellaneous group.

The ten patients with carcinoma of the stomach were all in the 40 to 70 years age group ; six were men. In eight patients clinical and x-ray diagnosis was confirmed by laparotomy. One patient had primary carcinoma of the transverse colon with direct malignant infiltration of the greater curvature.

Of the sixteen patients with peptic ulcer, fourteen were men: six were under 30, seven between 30 and 50, and three were more than 50 years of age. Diagnosis of duodenal, gastric, or gastro-jejunal ulcer was made by correlating clinical symptoms and signs with x-ray evidence following a barium meal. In ten cases laparotomy was performed and the diagnosis confirmed.

The three patients with " duodenitis " were all men under 30 years old. Diagnosis was made when classical symptoms of duodenal ulcer of recent origin were present, and when no x-ray evidence of peptic ulcer was found.

Of the four cases diagnosed as gastric neurosis, three were men: all were under 25 years of age. In three an anxiety state was present: the other was a case of depression with vague gastric symptoms following a head injury.

The three cases with lienteric diarrhoea were all young men: each gave a classical history of lienteric and post-pandrial diarrhoea of several years' duration. Clinical and x-ray examinations were negative.

The chronic dyspepsia-gastritis group was a heterogenous group of ten men and one woman. Four were under 30 years of age, three between 30 and 50, and the remainder older men. Three of the patients were chronic alcoholics and had morning anorexia with flatulent dyspepsia (one also had mild polyneuritis and liver cirrhosis) ; the remainder gave a history of dyspepsia (flatulence and/or discomfort after meals) with or without anorexia of some years' duration. In some cases the history was typical of chronic gastritis, but in others a satisfactory diagnosis could not be made. Cardiovascular disease was minimal or absent, and neither clinical nor x-ray examination was of positive help. In three cases, laparotomy revealed no abnormality.

The diagnosis of cholecystitis in one case (male, age 59 years) was made on clinical grounds and a negative barium meal radiograph: in the other (a woman, age 62 years), the barium meal radiograph showed diminished motility of the stomach, and gastroscopy an atrophic gastritis. At laparotomy, cholelithiasis was found and cholecystectomy and appendicectomy were performed.

The miscellaneous group were cases of subacute nephritis, repeated attacks of acute gastritis, achalasia of the cardia, and severe intestinal colic of unknown origin.

Results of N.R.E. tests.—Tests on twenty-nine of the fifty-three patients were within the normal range. The abnormal results fell into two groups, viz.: those in which there was no excretion of dye into the stomach (anexcretory type) (13 cases), and those in which the dye was poorly concentrated (11 cases). This group could be subdivided into two partially overlapping subgroups: (a) those with a normal E.T., C_M less than 2 and a normal or delayed C.T., and (b) tests with normal or delayed E.T. and C_M 1 or less. The dye excretion in three was slight and intermittent. For descriptive purposes, these will be called hypo-excretory types a and b. The figure portrays examples of

these curves in contrast with a normal result, and one in which the dye was not excreted.

Table I classified the tests according to diagnosis, the non-gastric controls being included for comparison. Table II is a more detailed case and test analysis of the patients with abnormal N.R.E. tests.

Of ten patients with gastric carcinoma, five failed to excrete neutral red ; in two excretion was poor and intermittent. Four of the eleven patients with chronic dyspepsia or gastritis did not excrete the dye, in two excretion was poor and delayed or intermittent. Both cases of cholecystitis were of the anexcretory type, but one patient also had atrophic gastritis. Of the sixteen peptic ulcer patients, ten had normal N.R.E. tests, the remainder hypoexcretory curves. Each case of "duodenitis" and lienteric diarrhoea, and three of the patients with gastric neurosis excreted neutral red normally. One of the latter (post head-injury depression) failed to excrete the dye. Of the four miscellaneous cases, the patient with achalasia of the cardia did not excrete neutral red and the patient with undiagnosed intestinal colic had only a very delayed and slight excretion of dye.

From these results it is clear that abnormal N.R.E. tests of the anexcretory and hypoexcretory types may occur in various organic disorders of the stomach and duodenum. Separate types of N.R.E. curves were not detected for particular diseases, and therefore the test cannot be of help in discriminating between (say) gastric carcinoma and gastritis. It is doubtful whether the test can distinguish between organic and functional disorder of the stomach, as cases of achalasia of the cardia (a local nervous disorder) and post-head-injury depression (with functional gastric symptoms) had anexcretory results. Moreover, normal N.R.E. tests can be given by patients with well-established organic disease of the stomach such as gastric carcinoma or peptic ulcer.

In practice the N.R.E. test is of value when an abnormal result is obtained : the conclusion may then be drawn that there is a disorder of the gastric mucosa of organic, functional, or possibly reflex origin.

Gillman (1944) found that normal results were repeatable after variable intervals of time. To confirm this, nine patients were subjected to a second test two to five weeks after the first. Seven had given normal N.R.E. curves on the first occasion and when repeated these tests were still within normal limits ; for example Case 3 (chronic gastritis) excreted neutral red at 3 minutes and concentrated it to a maximum of 2–3 in 14 minutes : five weeks later, excretion of dye began in 2 minutes and reached a maximum of 3 in 8

minutes. Two other patients failed to excrete the dye both at the first tests and when these were repeated a few weeks later.

Anatomy and physiology of neutral-red excretion.—Attempts were made to compare the blood level of the dye with its concentration in the gastric aspirate. In a patient with a blood volume of (say) 7 litres, the injection of 5 ml. of 1 per cent solution should produce a blood level of dye of about 1 in 140,000 or a plasma level of about 1 in 270,000 if all the dye remains in the plasma and if none diffuses into the extracellular tissues or is lost by excretion. *In vitro* experiments showed that when neutral red is added to oxalated whole blood, almost the entire quantity is present in the plasma, where its concentration could be estimated colorimetrically up to a concentration of 1 in 250,000 and detected up to 1 part per million.

Venous blood was removed from eight patients (from the arm opposite to the injection) 5 to 20 minutes after injection of the dye. In no case was it possible to detect neutral red in the plasma, although at the same time dye was being excreted in the juice in concentrations of 1/50,000 to 1/200,000. In two patients from whom blood was removed 5 and 6 minutes after injection, the dye was just appearing in trace concentration in the gastric juice. The urine was free from dye and it was considered impossible that excretion of dye could have been responsible for a plasma level of below 1 part per million. It was therefore concluded that neutral red rapidly diffuses into the extracellular tissues of the body, is present in a concentration of less than 1 in a million in the plasma, and is concentrated by the gastric mucosa during excretion at least 10 to 20 times.

In a patient with a duodenal ulcer (Case 49), the site of neutral-red excretion was established by a method suggested to one of us by Professor A. M. Boyd. This patient had an ulcer history of twenty years' duration, verified by barium meal x-ray examination. The fractional test meal showed hyperacidity and the N.R.E. test performed the day before the operation was normal (E.T. 2 minutes, C.T. 4 minutes. C_M 4).

Premedication with omnopon, 1/3 gr., and scopolamine, 1/150 gr., was given an hour and a half before operation which was carried out under ether anaesthesia. Partial gastrectomy was performed : during the course of the operation 5 ml. of 1 per cent neutral red was injected intravenously as soon as the greater curvature had been freed and the right gastric artery ligatured. Within a few minutes, the upper half of the gastric mucous membrane was stained red by the dye (see Plate

VIII) and there was a fairly sharp line of demarcation halfway down the stomach below which no dye was excreted. The anexcretory area included the lower half of the body of the stomach, the pyloric antrum, and the pylorus. Excretion of dye still persisted when the surface was washed with saline.

Was the lack of the excretion in the lower half of the stomach due to tying the right gastric artery and freeing of the greater curvature? To exclude this possibility the injection of dye was repeated several times during the course of further gastrectomies for peptic ulceration and the same distribution of dye excretion was obtained notwithstanding when the dye was injected or its relationship to the tying of vessels.

Was the lack of excretion in the lower half due to antral gastritis? The gastric mucosa looked normal and subsequent histological examination of various areas including the antrum showed no abnormality.

Evidence of the nervous control of dye excretion was obtained in other cases. In a patient with a gastro-jejunal ulcer (see Table II, Case 56) there was a low normal gastric acidity before operation, and excretion of neutral red occurred although this was delayed and rather poor. Four weeks after a successful transpleural vagotomy the tests were repeated. No excretion of dye was found, and the patient had achlorhydria.

N.R.E. tests with normal results were performed on five patients, who for various reasons were later submitted to sympathectomy: three patients had unilateral and two bilateral sympathectomy from T 5 to L 3 performed; splanchnicectomy was also carried out. The tests were repeated 2 to 3 weeks later and no significant changes in the excretion or concentration of dye by the stomach was found.

Several unsuccessful attempts were made both in rabbits and in man to discover the cellular origin of dye excretion. Biopsies of the stomach wall from the fundus, body and pylorus were taken within a few minutes of an intravenous injection of neutral red. The human material was collected during the course of partial gastrectomies. Frozen and paraffin sections were made and examined, stained and unstained, but no intracellular dye was seen even after treatment of the section with acid (neutral red is yellow in alkaline solution).

These results indicate that the excretion of neutral red is limited to the upper half of the gastric mucosa, that excretory activity is abolished by vagotomy, and that the sympathetic plays no part in the control of the excretion. The nervous control of the dye excretion and acid secretion is identical. Hydrochloric acid is secreted by the mucosa of the body of the stomach, probably over a wider area than that responsible for neutral-red excretion. The parietal (oxyntic) cells are not present in the pyloric region where an alkaline fluid is secreted. Stimulation of the vagus nerve in dogs produces a flow of strongly acid juice, whilst stimulation of the sympathetic splanchnic nerves gives only a slow steady secretion of fluid rich in mucus, often alkaline in relation (Pavlov, 1910; Carlson, 1923; Babkin, 1938). This latter secretion comes mainly from the pyloric glands and only to a minor extent from the body of the stomach.

From similar anatomical sites of acid secretion and neutral-red excretion, and from their identical nervous control, it might be expected on theoretical grounds that a relationship between acid secretion and dye excretion exists, and that an abnormality in one property would be accompanied by a parallel abnormality in the other, for example achlorhydria with failure to excrete the dye.

Relationship of acid secretion to neutral-red excretion by the gastric mucosa.—"Free" and "total" acid estimations were performed on specimens of gastric juice from all patients, including the controls. Forty of the cases with disturbed gastric or intestinal function had fractional test meals performed: in fifty cases acidity was estimated on several specimens of gastric juice obtained during the course of the N.R.E. test, and in four of these acid estimations were performed on juice obtained after an injection of histamine.

Acid secretion of the stomach has a wide range of normality, and it is therefore difficult to decide when abnormality begins. Arbitrary figures have to be adopted, and it was decided to accept a free acid equivalent to 70 ml. of N/10 hydrochloric acid per cent (70 per cent) as the limit of the upper range. Similarly the limit of the lower range was accepted as a free acid volume of 10 ml. of N/10 hydrochloric acid per cent (10 per cent). If one or more fractions of juice had a value of 70 per cent or over, *hyperacidity* was said to be present: if *all* the specimens of juice analysed were less than 10 per cent, *hypo-acidity* was said to exist.

From an examination of Tables I and II it is clear that the ability of the stomach to excrete neutral red and to secrete hydrochloric acid are not *necessarily* allied. Six patients (Cases 2, 9, 20, 25, 50, and 56) with normal acid production had hypo-excretory dye curves, one patient with hyperacidity excreted the dye poorly (Case 28), and another (Case 66) with normal acid production

failed to excrete the dye at all. Gillman's (1944) statement that patients with very poor or no excretion of dye are always achlorhydric is incorrect. However, there is a statistical correlation between normal acid production and normal neutral-red excretion and between achlorhydria and lack of excretion of dye. This relationship is probably the reflection of a common anatomical mucosal site for secretion and excretion and of a common nervous control of these activities.

Conclusions and Summary

The excretion of neutral red by the normal gastric mucous membrane follows the same pattern in different people within fairly narrow and easily recognized limits. Normality and abnormality in dye excretion is readily established using the neutral-red excretion test standardized by Gillman. When the test is repeated on the same individual, identical or closely similar results are obtained whether dye excretion is normal or abnormal. Rarely is an abnormal N.R.E. test given by a patient with a healthy gastric mucosa, and it can be said that abnormality in excretion of the dye is strongly suggestive of disordered gastric function.

For these reasons the N.R.E. test is superior and more reliable than the estimation of acid secretion. Other material advantages include safety, simplicity, and the short time it occupies. Furthermore, the dye and the apparatus are easily obtained, and if necessary the test can be performed by the clinician at the bedside.

All investigators agree that total failure to excrete neutral red is diagnostic of gastric dysfunction and many (Lourja and Mirkin, 1925: Morrison, 1938 ; Gillman, 1944) that partial suppression of dye excretion is an abnormal result preceding the final stage of total suppression. Whilst the present investigation is in the main in agreement with these observations, there is no doubt that patients with various disorders of the stomach and duodenum, including carcinoma and peptic ulcer, may have normal dye excretion. This may occur notwithstanding the duration of the disorder or the severity of the condition. A normal result is then of no value in distinguishing a normal from a pathological stomach, and the test is not nearly as sensitive as Gillman suggests.

Furthermore, neither a normal nor an abnormal result is of diagnostic use in differentiating between cancer, gastritis, and other conditions. Although the results of the peptic ulcer series suggest that complete suppression of excretion does not occur in cases of simple ulcer, this may not be true and may result from pure chance in the selection of the cases. Functional disorder of reflex or nervous origin may or may not suppress excretory function, so that the N.R.E. test is of no value in distinguishing organic from non-organic disease.

The problem of serial tests on the same patient to determine the deterioration or recovery from gastric disorder was not studied.

Although a statistical relationship exists between excretion of neutral red and secretion of acid, the correlation has exceptions and in any individual case disorder of one function may or may not be accompanied by a parallel disorder of the other.

Finally, experiments and observation indicate that, following the injection of neutral red, most of the dye rapidly diffuses into the extracellular tissue fluid and is present in the blood in minute undetectable traces (concentration < 1 part per million) although at the same time it is being excreted by the gastric mucosa in concentrations up to 1 in 50,000. The parasympathetic vagus nerves are probably the excitory nerves controlling dye excretion, and the sympathetic plays no part in the activity.

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THE FEULGEN REACTION APPLIED TO CLINICAL HAEMATOLOGY

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The Feulgen reaction is a cytochemical test for the detection of desoxyribonucleic (thymonucleic) acid. After acid hydrolysis, desoxyribonucleic acid in the presence of sulphurous acid gives a characteristic red-purple colour with basic fuchsin, and the depth of the colour roughly reflects the concentration of the compound. The detailed chemistry of the reaction has been fully described by Baker (1942). The reaction has been known for fifteen years and is much used in biology, especially in genetics, but it has only recently been applied to the problems of clinical haematology: La Cour in 1944 used the reaction to study mitotic abnormalities that occur in the bone marrow cells in pernicious anaemia ; Thorell working in Caspersson's laboratory in Stockholm used the Feulgen reaction together with ultra-violet absorption techniques for studying the intimate metabolism of nucleoproteins in blood-forming cells (Thorell, 1944, 1947). We have utilized the Feulgen reaction to give help in classifying blood and bone-marrow cells when the differentiation is of clinical importance and when the routine methods fail to show really clear separation between the different types of cells.

Details of Technical Method

The technique is a slight modification of those proposed by Rafalko (1946) and Darlington and La Cour (1942).

Air-dried smears of blood or bone-marrow are used and should be fixed as soon as possible, not more than one hour after preparation. The bone-marrow is obtained by sternal biopsy. The fixing solution is composed of: methyl alcohol 15 parts, 5 per cent acetic acid 5 parts, formalin (40 per cent formaldehyde) 1 part, and water 5 parts. The smears are immersed in this fixative for 10 minutes and then treated as follows:

1. Wash in tap water for 10 or 20 minutes.

2. Wash in distilled water for 2 minutes.

3. Place in N/HCl at room temperature for 2 minutes, then (4) in N/HCl at 60° C. for 8 or 10 minutes, and (5) rinse with N/HCl at room temperature.

6. Rinse with distilled water.

7. Stain with leucobasic fuchsin solution (*vide infra*) for 1½ to 2 hours.

8. Put through two or three changes of SO_2 water (*vide infra*) for 1 or 2 minutes.

9. Wash in tap water for 10 to 15 minutes.

10. Then dehydrate by immersing in 40 per cent, 80 per cent, and finally absolute alcohol for 5 minutes each.

11. Clear with xylol.

12. Mount in a neutral synthetic mounting medium.

The leucobasic fuchsin is prepared as follows: a 0.5 per cent solution of basic fuchsin is decolorized by bubbling SO_2 through it for 1 hour ; if decolorization is not then complete, activated charcoal (1 g. to 100 ml.) should be added to it, and it is shaken and filtered.

The SO_2 water is prepared by bubbling SO_2 from a syphon through distilled water until a saturated solution is obtained.

It is sometimes helpful to counterstain with Jenner's stain to outline the cytoplasm and stain cytoplasmic granules. This can be done without interfering with the Feulgen stain. The Jenner's stain then replaces stage 10 of the technique.

Nuclear Appearances of Blood Cells when Stained by Feulgen's Reaction

With this stain the erythrocytes disappear ; the leucocytes and erythroblasts take up stain according to the concentration of desoxyribonucleic acid present in the nucleus ; immature cells have a relatively pale coloration, mature cells a deep tint ; the cytoplasm does not stain. The pale-stained cells are said to be Feulgen-negative, and the deeply-stained cells Feulgen-positive. Nucleoli show up well as clear spaces in the nucleus with a
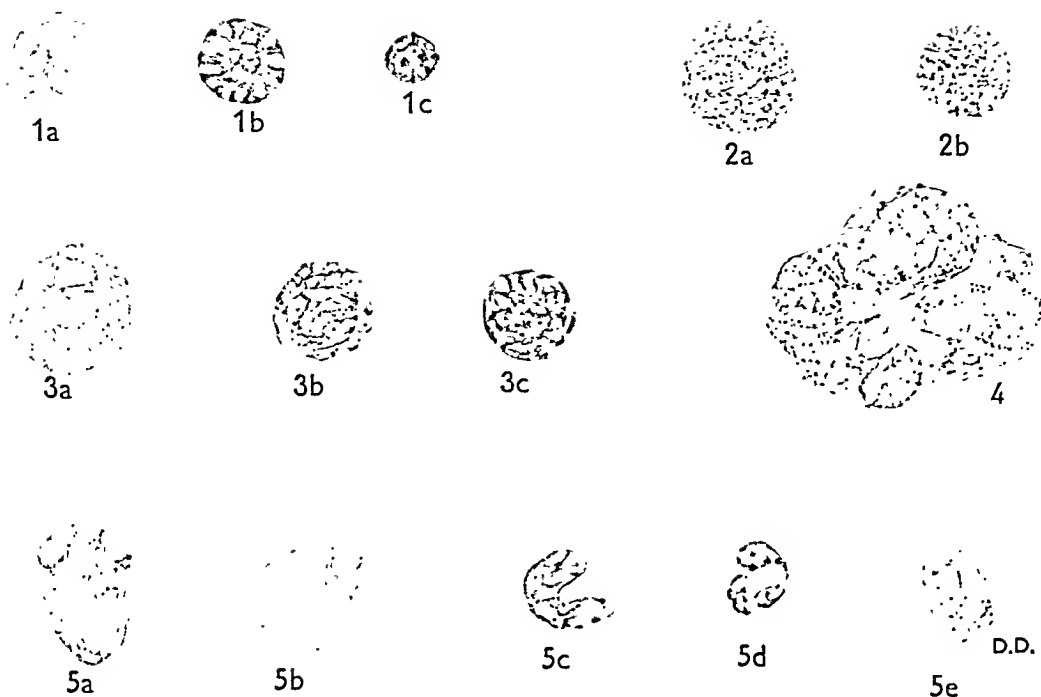
BONE-MARROW CELLS STAINED BY THE FEULGEN TECHNIQUE

1.—Lymphocytes from a case of chronic lymphatic leukaemia : (a) Lymphoblast, Feulgen-negative, fine chromatin pattern, two nucleoli ; (b) Immature lymphocyte, Feulgen-positive, but shrunken nucleoli still present; (c) Small mature lymphocyte, Feulgen-positive, with chromatin concentrations round the periphery.

2.—Megaloblasts, untreated pernicious anaemia : (a) early megaloblast—note network arrangement of chromatin and absence of nucleoli; (b) intermediate megaloblast, coarser pattern but network still distinct.

3.—Normal erythroblasts : (a) Pro-erythroblast, Feulgen-negative ; note large nucleolus and fine but distinct chromatin pattern ; (b) early normoblast, moderate Feulgen reaction ; note coarse concentration of chromatin and absence of nucleoli ; (c) intermediate normoblast, large size, Feulgen-positive with lumpy chromatin pattern.

4.—Megakaryocyte, mature cell moderately Feulgen-positive; no nucleoli.

5.—Granular leucocyte series from chronic myeloid leukaemia : (a) typical myeloblast, Feulgen-negative, four nucleoli ; (b) pro-myelocyte, still Feulgen-negative, only one nucleolus; (c) metamyelocyte, still only moderate Feulgen staining ; nucleoli have disappeared ; (d) mature polymorphonuclear granulocyte, Feulgen-positive streaks ; (e) micromyeloblast : note relatively pale Feulgen stain, fine chromatin pattern and the presence of nucleoli.

relatively deep staining rim which is more prominent in the more mature cells. The appearances of individual cells are as follows:

Granulocytes.—In the *myeloblast*, the nucleus takes on a pale stain but the chromatin pattern can often be clearly distinguished; there are two to four large nucleoli clearly detectable as colourless spots. The *micromyeloblast* is rather more deeply stained but nucleoli are clear. The *promyelocyte* and *myelocyte* show changes indicating increasing maturity—the nucleoli decrease in number and size and show a thin surrounding layer of Feulgen-positive material. The *metamyelocyte* has no nucleoli,.but is not so deeply stained as the mature *polymorphonuclear*, which has Feulgen-positive coarse chromatin with two or three prominent positive spots, usually one in each lobe of the nucleus.

Lymphocytes.—The *lymphoblast* has a Feulgen-negative nucleus of fine structure, not very clearly defined, and there are one or two prominent nucleoli. The *immature lymphocyte* has a coarser and more deeply staining nuclear chromatin, but nucleoli are still present though small. The nucleus of the *mature lymphocyte* is composed of Feulgen-positive blocks of chromatin arranged mainly around the periphery.

Monocytes.—The mature monocyte has a fine chromatin structure and stains a faint pink. No aggregations of chromatin occur, and there are no nucleoli.

Erythroblasts (Nomenclature according to Israëls, 1941).—The *pro-erythroblast* shows a distinct chromatin network but is Fuelgen-negative; nucleoli are prominent and are distinguished by a deeply stained surrounding layer.

The *normoblasts* show early appearance of Feulgen-positive material; even the early stage is quite deeply stained and shows very well the coarsening and clumping of the chromatin strands. The intermediate normoblasts are fully Feulgen-positive and show the typical coarse chromatin structure.

In the *megaloblast* series the fine network nuclear structure shows up very strikingly when this technique is used. The early stages show only moderate Feulgen staining; the nuclear strands of the intermediate stage are fully Feulgen-positive.

Megakaryocytes.—The large nucleus of the mature cell is moderately Feulgen-positive and no nucleoli are present.

Discussion

The Feulgen reaction has thus several uses as a supplementary technique in clinical haematology.

1. It gives a clear decision about the stage of maturity of a given cell. This is very important in the leukaemias. A not uncommon cause of incorrect diagnosis is confusion between small myeloblasts (micromyeloblasts) and lymphocytes. Sometimes with ordinary stains the differentiation is very difficult even with a good technique; in particular, nucleoli are often obscured by Romanowsky stains. Schulten (1937), for example, shows on Table 13 in his Atlas a group of cells that he cannot clearly define as myeloblasts or lymphocytes. The Feulgen stain distinguishes clearly between the Feulgen-negative myeloblasts with well-defined nucleoli and the Feulgen-positive lymphocytes without nucleoli.

In chronic myeloid leukaemia the presence of myeloblasts can be used to assess the progress of a case and response to treatment; if these immature cells are few and their proportion decreases with treatment, the prognosis is good. It is much more difficult to assess a case of lymphatic leuleuamia in this way, because with Jenner-Giemsa stain it is not a simple matter to detect immature lymphocytes. The Feulgen stain provides the necessary information, as the proportion of negative or weakly positive lymphoblasts and immature lymphocytes with detectable nucleoli is readily determined and these cells contrast well with the Feulgen-positive mature lymphocytes without nucleoli.

2. The characteristic difference between normoblasts and megaloblast is in the nuclear structure, the chromatin being arranged respectively in clumps or in network of strands. The Feulgen stain gives a sharper picture of the intimate nuclear structure than is possible with Jenner-Giemsa, and another difference is that the normoblasts become more deeply stained at an earlier stage of development than the megaloblasts.

3. The differentiation between lymphoblasts and myeloblasts is a problem as old as clinical haematology, and many techniques—dark-ground illumination, supravital staining, oxidase stains—have been applied with indifferent success. The Feulgen reaction helps because it defines nucleoli. Naegeli (1923) pointed out that, whereas lymphoblasts have few nucleoli, in myeloblasts they are usually multiple. In our experience there are usually one or two nucleoli in lymphoblasts, rarely three; whereas in myeloblasts there are two to four nucleoli, most commonly three. Nucleolus-counting can be a difficult task with Jenner-Giemsa-stained preparations; with Feulgen stain it is an easy matter to see whether the majority of the immature cells have few or many nucleoli. The presence of a majority of cells with three or

more nucleoli suggests a myeloblastic form of leukaemia.

The nucleoli with their rim of dark-staining material are a typical feature of the Feulgen-stained cells. They appear often much larger than in the Jenner-Giemsa stained preparations. Thorell has related the number and size of the nucleoli to the amount of cytoplasm that is to be formed by the cell in the course of development. The myeloblasts of acute leukaemia often have numerous and prominent nucleoli, yet fail to develop cytoplasm. Two possible explanations are that the cells are abnormal, like malignant cells, and do not develop normally; or that they are normal cells in an abnormal environment. In support of the latter explanation are Israëls' (1940) observation that leukaemic myeloblasts in culture could mature into myelocytes, and Schwind's (1947) claim that injection of normal blood plasma tends to decrease the proportion of myeloblasts in the blood. It is therefore likely that in pathological states the connexion between nucleolar mass and cytoplasm formation is disturbed.

Summary

1. Details are given of a rapid technique for applying the Feulgen reaction to blood and bone-marrow cells.

2. The appearances of the more important blood and bone-marrow cells when stained by this Feulgen technique are described and illustrated.

3. The Feulgen reaction is a valuable supplementary technique in clinical haematology for the following reasons:

(a) It distinguishes between immature and mature cells, especially between lymphocytes and micromyeloblasts, and it can be used to assess the proportion of immature cells in lymphatic leukaemia.

(b) The nuclear differences between normoblasts and megaloblasts are sharply defined.

(c) It defines nucleoli very clearly and can therefore aid in distinguishing lymphoblasts with one or two nucleoli from myeloblasts with two to four nucleoli.

4. The suggested correlation between number and size of nucleoli and subsequent cytoplasm formation appears to be disturbed in disease.

The plate was painted by Miss D. Davison, medical artist to the University.

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HAEMOSTASIS WITH AN EASILY PREPARED STABLE THROMBIN SOLUTION

BY

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During physiological blood coagulation after injury the "clotting enzyme," thrombin, is always formed in excess. Such large amounts of liberated thrombin would ultimately cause extensive intravascular thrombosis, were it not for the presence of substances with antithrombic activity, which destroy the thrombin formed, partly before and partly after its action upon fibrinogen. While these substances are of great protective importance in physiological coagulation, they have hampered *in vitro* work considerably. Much difficulty has been caused by them in the quantitative determination of prothrombin, because it has not been possible to convert prothrombin to thrombin completely in plasma or whole blood and to measure thereafter the activity of the latter in clotting fibrinogen. An undeterminable part of the thrombin formed has always been destroyed by antithrombic activity.

In the same way, the *in vitro* isolation of thrombin for the purpose of haemostasis has been hampered by such destruction of thrombin immediately after its formation. While American authors (Cohn and others, 1940, 1946 ; Edsall and others, 1944 ; Milstone, 1942 ; Seegers, 1940 : Seegers and McGinty, 1942 ; Seegers and others, 1938, 1945) have solved this problem by the isolation, instead of thrombin, of its precursor, prothrombin, and by subsequent elaborate purification of the latter to separate it from as much antithrombin as possible, yet they have not been able to remove all the antithrombin, and this is shown by the fact that their preparations are not very stable in the liquid state.

Recently it has been found that the antithrombic activity of plasma can be specifically suppressed by dilute ethyl alcohol (Sternberger, 1947), and the

effect was made use of for the development of a simple two-stage method for the determination of prothrombin that is independent of any inactivators and that is thus able to determine prothrombin quantitatively (Sternberger, 1947, 1948). In the present communication preliminary experiments are reported upon the isolation and clinical use of thrombin from blood in which the antithrombic activity has been suppressed and prothrombin subsequently converted to thrombin. It will be shown that this preparation offers the following advantages over previous ones:

1. The thrombin obtained is completely free from antithrombin, not only separated from it by fractional purification as in previous methods. Therefore the thrombin is stable for many months, in the liquid state, diluted or undiluted. Other thrombin preparations are not stable once they have been diluted (Bailey, 1945 ; Glazko, 1947).

2. No elaborate purifications are necessary in the preparation of our thrombin. The procedure is, therefore, so simple that any laboratory can produce its own thrombin for surgical haemostasis. No complicated equipment is necessary.

3. During its preparation our thrombin is co-precipitated with acacia. The presence of acacia gives the thrombin a sticky consistency. This makes it possible to use thrombin solutions effectively with much smaller activity than hitherto, derived from a correspondingly smaller amount of blood.

Method of Preparation of Thrombin

Step 1: "Thrombinization."—A temperature between 16° and 22° C. (60.8° to 71.6° F.) is maintained while the following ingredients are placed

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successively into a flask and stirred after each addition:

380 parts of 50 per cent by volume of ethyl alcohol in normal saline solution.

145 parts of normal saline solution.

25 parts of 0.2 M calcium chloride solution.

210 parts of human blood (whole blood ; 9 parts of blood obtained by venepuncture and rendered incoagulable by addition to 1 part of 0.1 M sodium oxalate solution).

75 parts of human milk.*

75 parts of 50 per cent by volume of ethyl alcohol in normal saline solution.

The material obtained after the lapse of about 5 to 10 minutes (crude thrombin) will clot an equal volume of human plasma in 6 to 8 seconds. It is very stable. It may be processed immediately or may be kept in the refrigerator without loss of activity for at least eight months.

The subsequent steps may be performed at a temperature between 16° and 28° C. (60.8° to 82.4° F.).

Step 2: Denaturation of labile blood proteins.— To 32 parts of " crude thrombin " (shaken well to obtain a uniform suspension) are added 18 parts of 95 per cent ethyl alcohol by volume. The whole is shaken violently and centrifuged immediately. The supernatant fluid is decanted, and Step 3 immediately proceeded with.

Step 3: Elution.—The sediment, stirred well to break it up as completely as possible, is again dissolved in 32 parts of a 10 per cent solution of powdered acacia in normal saline and centrifuged.

Step 4: Co-precipitation.—To 30 parts of the supernatant fluid are added 32 parts of 95 per cent of ethyl alcohol by volume and the whole is well mixed and centrifuged. The supernatant fluid is decanted and the next step immediately proceeded with.

Step 5: Resolution.—The sediment is dissolved in 3 parts of normal saline solution. This is done by wiping the sticky sediment around a glass rod and breaking it up by pressing it at the same time against the walls of the centrifuge tube. It will then dissolve easily in the relatively small volume of added liquid.

We usually dissolved the sediment of thrombin and acacia in a solution of 1:1,000 merthiolate (sodium

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ethyl mercurithiosalicylate) in normal saline. When thrombin was being prepared for surgical use, sterile technique was employed from the moment of the second addition of alcohol on (in Step 4) (sterilization by 50 per cent alcohol). In this case the final thrombin and acacia solution was dissolved in 1 : 7,500 merthiolate in normal saline solution. All our thrombins so prepared were tested for sterility, and in none did any growth take place.

Results

The Table gives the clotting times obtained with our acacia-containing thrombin solution. No loss of activity of the thrombin solution took place during a period of six months' storage in the ice box.

TABLE

CLOTTING TIME WITH ACACIA-CONTAINING THROMBIN SOLUTION

Dilution of thrombin (after addition to test plasma)	Clotting time (seconds) (assay by adding 0.2 ml. of diluted thrombin to 0.2 ml. of oxalated human plasma)
1 : 2	2 1/2
1 : 6	2
1 : 10	5 1/2
1 : 20	10
1 : 40	18

Clinical Use

Because of the admixture with acacia, the thrombin preparation, when applied to a wound, will adhere to the bleeding surface. It does, therefore, as shown by clinical experience, prove effective as a haemostatic agent, although its *in vitro* activity is less than that of some other preparations of thrombin, this corresponding to the smaller amount of blood required for its isolation.

Our thrombin can be applied by simply placing a few drops of the gelatinous solution upon a plug of gauze. The stickiness of the acacia prevents the thrombin from being soaked up by the gauze, the solution tending to adhere to its surface. Thus, it produces an effective haemostatic layer between gauze and bleeding wound. The fact that the preparation is not soaked up by the gauze also adds to the economy of its use. The gauze covered with thrombin-acacia is applied to the wound until haemostasis is produced. It may then be removed. While a clot is produced at the interface between the thrombin-acacia and the bleeding surface, this

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* Many samples of blood carry with them enough thromboplastin for making it unnecessary to add further thromboplastin for the conversion of prothrombin to thrombin in the presence of ethyl alcohol. But the occurrence of sufficient amounts of thromboplastin in the blood is very irregular, so that it becomes necessary to use additional thromboplastin to obtain a dependable preparation of thrombin.

In this preparation of thrombin from human blood *human* milk is used. It has been claimed that foreign protein in thrombin preparations would sensitize the patient. However, conclusive proof of this warning is lacking.

Cow's milk also contains thromboplastin, but its activity is much smaller than that of human milk.

clot—because of the stickiness of the preparation —does not extend beneath the surface of the gauze, and does not, therefore, cause fresh laceration and fresh bleeding when it is subsequently removed (a difficulty encountered by Tidrick and others (1943) in the use of their preparation with cotton plugs and bits of gauze). Bleeding also recurs, as reported by Bailey and others (1945), if fibrin foam (Cohn and others, 1940, 1946 ; Edsall and others, 1944) applied to a wound with thrombin is removed after production of haemostasis.

The stability of our preparation in the liquid form makes it possible to use a few drops of thrombin for one purpose, and to store the remainder of the bottle in the refrigerator until new requirements arise. This further adds to the economy of the preparation, in contrast to those products that are supplied in a powdered form and cannot be stored after once having been dissolved. Even a few hours' storage at subtropical room temperature (26° to 28° C. (78.8° to 82.4° F.) in our laboratory and hospital at the time this preparation was being developed and tried clinically) does not impair the effectiveness of our preparation. The thrombin prepared by Cohn and his team is rapidly destroyed at temperatures higher than room temperature (Bailey and others, 1945).

The thrombin described here has been used by Dr. Lachmann, Chief of the Otolaryngology Department of this hospital. He reports as follows:

"The thrombin was used in twenty tonsillectomies and in eight Caldwell-Luc's operations on the maxillary sinus. In tonsillectomies, at the completion of the operation a tampon of gauze covered with a few drops of thrombin solution was applied with slight pressure for 1 to 2 minutes to the tonsillar bed. It was then removed. At this time the wound was dry in every instance, including cases in which bleeding before application of thrombin was very strong. Only spurting blood vessels were ligated before using the thrombin. Many cases would have required careful ligation, had thrombin not been used.

In Caldwell-Luc's operation on the maxillary sinus, 1 to 3 ml. of thrombin were instilled upon completion of the operation. Bleeding always stopped within a short time (2 to 5 minutes). The cavity remained without tampon. Only in one case, which showed very heavy haemorrhage because of hypertension (270/140 mm. Hg). the cavity was packed lightly with gauze upon which thrombin had been placed. This stopped the haemorrhage

at once, without recurrence upon removal of the tampon the following day."

The thrombin was also used in a case of dicoumarol hypoprothrombinemia (Sternberger, 1948) (prothrombin: 12.5 per cent determined by the stabilized thrombin method (Sternberger, 1947)). The patient started to bleed from the operation wound eleven days after the second stage of Lahey's abdominal-perineal resection of the rectum (Dr. Joseph). Haemostasis with thrombin was immediate.

Summary

The principle of the suppression of the antithrombic activity of plasma by ethyl alcohol was applied to the development of a new method for the isolation of thrombin. The simplicity of the method makes it possible for any clinical laboratory to prepare thrombin for its own use for surgical haemostasis. The thrombin prepared is stable in the liquid form. The clinical use of the preparation in haemorrhage and the advantages of admixture of acacia with thrombin have been discussed.

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SEROLOGICAL ASPECTS OF WEIL'S DISEASE

BY

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The agglutination test for Weil's disease (leptospirosis icterohaemorrhagica) is essentially the same as other serological procedures. The disease, however, is comparatively uncommon, and the leptospira suspensions are relatively unstable. For these reasons there has been a tendency for work on leptospirosis to be concentrated in a few laboratories. The observations recorded here are based on the findings in agglutination tests carried out on serum from 219 cases of leptospirosis and from 875 patients suffering from other diseases. Epidemiological and clinical notes on some of these cases have been described by Broom and Alston (1948).

Serological Methods

Agglutination of formalinized cultures of *Leptospira icterohaemorrhagiae* was the diagnostic test used in this work. Some workers prefer suspensions of living organisms, but the need to have well-grown, active cultures always available is a disadvantage in routine work, and in our experience the two methods are equally sensitive. Furthermore, with either fresh or inactivated serum, formalinized suspensions agglutinate in the same manner over the whole titre, whereas living cultures lyse in some dilutions of serum, and thereafter present a series of varying agglutination pictures at the different serum-concentrations. In addition, a single strain of leptospira may be lysed by one serum and agglutinated by another, in the same dilutions. All these variations are of interest and value for research purposes, but they introduce unnecessary complications for clinical diagnosis.

Antigen.—The same strain of *L. icterohaemorrhagiae* was used throughout, although parallel tests were often carried out with other strains. This strain, "Jackson," was isolated in 1935 from a human case of Weil's disease, and contains both the A and B antigens described by Petersen (1939). Though now completely non-virulent for guinea-pigs, it provides a satisfactory agglutinating suspension.

Stock cultures are maintained in Fletcher's (1927) agar medium and are used to inoculate flasks of Fletcher's broth (in which broth is substituted for agar) or of Korthof's (1932) medium. The cultures are incubated at 26° C. for five to seven days until they reach a density of about 10^7 leptospirae per ml. In practice, no count is made; the density is judged by examining a drop of culture by dark-field illumination with a 16-mm. objective and a × 10 ocular. Sufficient formalin is added to give a final concentration of 0.2 per cent; the suspension is centrifugalized, and any deposit is discarded.

The stability of formalinized suspensions of leptospirae is very variable, and the responsible factors are not yet known. It is our custom, therefore, to prepare quantities of only 50 or 60 ml. at one time, and to renew the suspensions at intervals of a few weeks. The suitability of the new suspension is determined by testing the titres of known positive sera in parallel against old and new suspensions. If the new culture differs in agglutinability to an appreciable extent, it is discarded. No attempt has been made to work out a factor for different suspensions, as is done, for example, with standard agglutinating suspensions of the enteric group of bacteria.

Agglutination test.—The technique used is that described by Schüffner and Mochtar (1927). Mixtures of serum and antigen are prepared so that the final dilutions of serum are 1/10, 1/30, 1/100, 1/300, etc., and interaction is allowed to continue overnight at room temperature or at 5° C. A single drop of each dilution is examined, without a coverglass, by dark-ground illumination, using a 16-mm. objective and a ×12.5 ocular. In our opinion this is the best method of reading the result, because this magnification allows true agglutination in low dilutions (1/10 and 1/30) to be distinguished from the non-specific clumping which is produced by a small proportion of sera. In true agglutination, loose floccules of leptospirae are seen

against a background containing no free organisms, whereas non-specific clumping shows small, compact, refractile masses in which only a proportion of the leptospirae have become entangled, leaving the majority free in the field. For confirmation in doubtful cases the specimen is examined with a 2-mm. objective.

Specificity of the Reaction

Most observers would agree with Smith and Davidson (1936) that " the sero-reaction is entirely specific, agglutinins and lysins being formed as a result of leptospira infection only." These observers found no agglutination in serum dilutions, of 1/10 in a series of 403 control tests. Stuart (1946) states that he has " never found antibodies at a titre of 1/10 in people who had not been exposed to known or highly probable leptospiral infection." Ward and Turner (1942), in a survey of workers in different trades, accept a titre of 1/10 as evidence of old infection.

Evidence of a more direct nature is available from the work of Alston and Brown (1935), who tested sera from sewer-workers and found that samples with agglutination titres of 1/100 would protect guinea-pigs against infection with virulent L. icterohaemorrhagiae. The subjects were in normal health when the blood samples were taken, and the agglutination was presumed to be due to residual antibodies from past infection. Similar results were reported by Mason (1938), who obtained partial protection by serum showing a titre of 1/30, and complete protection by sera with titres of 1/100 and 1/300. Our experience of the agglutination test is, in the main, in agreement with these opinions. In this series there were 875 patients where the final diagnosis was not Weil's disease. Of these, nine sera gave agglutination in dilutions up to 1/30, and a further fourteen in a dilution of 1/10 only. Most of these patients worked at occupations where Weil's disease is a recognized hazard, for example on farms, in coal mines, or in sewers, so the possibility of previous contact with leptospirae could not be ruled out.

Para-agglutinins

Although a positive agglutination test is a specific sign of infection with leptospirae, there is some serological overlap between L. icterohaemorrhagiae and the dog leptospira, L. canicola, which also is infective to man though only to a minor extent. In most instances the titre of a serum is 10 to 100 times higher for the homologous species, but occasionally, in the early stages of the disease, the titres run parallel, or may even be higher with L. canicola. According to Gispen and Schüffner (1939) this is more likely to happen when the infecting leptospira is of the " incomplete " type of Petersen. An example of this effect is shown in Table I, which sets forth the titres against the two leptospirae of specimens of serum taken on the sixteenth and twenty-sixth days of illness. An earlier specimen, of the eighth day, was negative with both leptospirae. Absorption tests on the sample of the sixteenth day showed the infection to be due to L. icterohaemorrhagiae, but the strain was not isolated to test whether it was of the incomplete type.

Time of Appearance of Antibodies and Titres Reached

A certain number of observations have previously been made on the interval which elapses between the onset of symptoms and the appearance of demonstrable agglutinins in the serum, and also on the manner in which the antibody titre rises during the course of the disease. In the opinion of Postmus (1933) no positive result is to be expected until after the sixth day; reactions then occur in low dilutions (1/10 to 1/25), and

TABLE I

AGGLUTINATION OF *L. ICTEROHAEMORRHAGIAE* AND *L. CANICOLA* WITH WEIL'S DISEASE SERUM

	Dilution of serum							
	1/10	1/30	1/100	1/300	1/1000	1/3000	1/10,000	
L. icterohaemorrhagiae ..	+	+	+	+	÷	±	—	Serum of 16th day
L. canicola	±	±	+	+	÷	+	—	
L. icterohaemorrhagiae ..	+	+	±	+	÷	+	—	Serum of 26th day
L. canicola	+	+	±	—	—	—	—	

+ = agglutination; ± = partial agglutination; — = no agglutination.

TABLE II

RELATION BETWEEN DAY OF DISEASE AND APPEARANCE AND DEVELOPMENT OF ANTIBODIES

					Day of disease				
2	3	4	5	6	7	8	9–13	14–20	<20
0							300		
	0					300			
	0						1,000		
		0				100		300	
		30						10,000	
			0					300	1,000
			0					300	3,000
			0	0	_10		1,000	3,000	
			0				1,000	3,000	
			30				1,000		
			100				3,000		
			300					3,000	
				0					1,000
				0					3,000
				0					3,000
				0			300		10,000
				10			1,000		
				30			100		
				30			3,000		
				30				3,000	
				30			1,000		30,000
					0			1,000	
					0			1,000	
					10			300	3,000
					30				10,000
					100		300	1,000	3,000
					100			3,000	3,000
					300			3,000	3,000
					1,000			3,000	
						0		3,000	30,000
						30	1,000		
						30		3,000	
						100		1,000	
						100		3,000	
						100			10,000
						300		1,000	
						300	1,000	3,000	
				0			0	100	300
			0				0		30,000

0 = No agglutination at dilution of 1/10.   10, 30, etc. = Reciprocals of agglutination titres.

the titre rapidly rises to reach a maximum of 1/10,000 to 1/100,000 by the end of the third week. Kisker (1935) considers that agglutinins are not present before the eighth day, when titres of 1/160 to 1/300 are obtained; thereafter a maximum of 1/40,000 to 1/80,000 is reached between the fifth and seventh weeks. Mochtar and de Reede (1941) also found the agglutination test negative during the first week; the highest titres in their series occurred during the third and fourth weeks.

We have carried out tests at the relevant times on thirty-nine patients, and the results are set out in Table II. The wide variation in the day on which antibodies appear, the rate at which the titre rises, and the maximum reached, is very striking. The earliest positive agglutination was recorded on the fourth day after onset, but negative as well as positive results were obtained on all days up to the eighth, and two sera were still negative on the twelfth and thirteenth days respectively. In some instances, of course, the onset may have been gradual and the stated day may not be quite accurate, but the disease began abruptly in the patient whose serum gave a positive result on the fourth day.

As will also be seen from the table, there is no apparent correlation, either positive or negative, between the time of first appearance of antibodies and the final titre. On the average, the titres seem to be lower than those often recorded, and we have never found anything approaching, for instance, the 1/2,000,000 reported by Senthille and others (1946). We have tested serum from 169 patients during the third week of illness or later, and the highest titres reached are shown in Table III. Only about 20 per cent rose to 1/10,000, and 7 per cent as low as 1/300.

TABLE III
HIGHEST TITRE OF 169 SERA TESTED DURING THIRD OR LATER WEEK OF DISEASE

	Reciprocal of titre				
	300	1,000	3,000	10,000	30,000
No. of sera with titre ..	12	47	73	30	7

Effect of Therapeutic Serum

In some cases the question arises whether the administration of therapeutic serum has vitiated the result of a test. Alston (1940) found that the agglutination became negative in rabbits within three days after the injection of 10 ml. of antileptospiral serum per litre of plasma. We tested the result in the human case of influenza, described by Robertson (1946), who received approximately 40 ml. of immune horse-serum per litre of plasma. The agglutination titre of the horse serum was 1/30,000, which would give a calculated titre of about 1/1,000 in the general circulation. Samples of blood taken five and sixty minutes after administration showed a titre of 1/300. The level fell to 1/100 after twelve hours, and to 1/30 after twenty-four hours.

Persistence of Antibodies

It has been shown by a number of workers that antibodies can be demonstrated in the serum many years after recovery from an attack of Weil's disease. Thus Kisker (1935) found positive agglutinations from two to sixteen years after recovery ; Uhlenhuth and Fromme (1930) after twenty-two years ; and Stuart (1939) after twenty-eight years. We have had few opportunities to examine serum after recovery, and the longest interval was four years, but agglutinins were present in all cases. The practical importance of these residual antibodies will be referred to later.

Discussion

A number of factors must be taken into account when the clinical significance of the results of leptospiral agglutination tests is being assessed. Although in general the test is positive about the end of the first week, there are occasions when the appearance of agglutinins is delayed, as in the two cases cited above which still reacted negatively on the twelfth and thirteenth days respectively. A negative finding may thus merely mean that the blood was examined before antibodies were present. Multiple negative tests throughout the course of illness will exclude Weil's disease with considerable certainty, because it must be very rare to find cases such as that reported by Garnier and Reilly (1917), in which, although leptospirae were isolated from the patient, no antibodies appeared in the blood.

Positive agglutination reactions may be the result of (1) the administration of immune serum ; (2) the presence of residual antibodies from past infection ; (3) present disease.

The giving of serum might affect the result of the test in the early stages, and should be reported by the physician who sends blood for examination.

The possibility of past infection is important in patients who have been in contact directly or indirectly with rats. McKeon and Brown (1936) describe an attack of infective hepatitis in a miner, where the diagnosis was complicated by a positive leptospira agglutination. Three similar cases have occurred in this series. The patients, a farm worker, a seaman, and a refuse collector, all suffered from jaundice. Each case showed a positive agglutination titre of 1/100 or 1/300 but, as there was no increase at subsequent tests, the illness was obviously not Weil's disease.

When the clinical picture is suggestive, the results shown in Table III indicate that a positive agglutination in serum dilutions of 1/1,000 or more may be accepted as confirming the diagnosis. The interpretation of a lower titre is difficult if the findings of only one test are available. As is shown in Table II, titres as low as 1/300 or even 1/100 may occur quite late in the disease. In two of these patients the diagnosis was proved correct by isolating leptospirae from the urine. Low readings cannot therefore be automatically regarded as resulting from past infection. The correct decision can be reached only by repeated examinations.

Early in the disease, say up to the fourteenth day, we would consider positive agglutination even to the lowest titres to be suspicious, and recommend the testing of further specimens.

Summary

1. A description is given of the material and methods used in applying the leptospira agglutination test to serum from 219 cases of Weil's disease and 875 of other illnesses.

2. Evidence is put forward confirming the specificity of the test.

3. The shortest interval after the onset of symptoms at which antibodies were detected in the serum was four days. Both positive and negative reactions occurred up to the eighth day ; two sera which later became positive were still negative on the twelfth and thirteenth days respectively.

4. Examples are given of the variability of the rate of production of antibodies, and of the highest titre reached in individual patients.

5. In a single experiment samples of serum were tested from a subject who had received 40 ml. of antileptospira serum per litre of plasma. From an initial titre of 1/300 at five minutes, the antibodies fell to 1/30 at twenty-four hours

6. In discussing the clinical interpretation of agglutination tests, stress is laid on the need to consider the result in conjunction with the length of time after infection, and to bear in mind the possibility of previous contact with infection.

7. With suggestive clinical symptoms, an agglutination titre of 1/1,000 is considered to confirm the diagnosis. With lower titres and in doubtful cases repeated tests are recommended.

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SALMONELLA BIRKENHEAD: A NEW SALMONELLA TYPE CAUSING FOOD-POISONING IN MAN

BY

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During the six weeks from mid-July to the end of August, 1947, nine strains of a new *Salmonella* type were received from nine patients living in different parts of England, five of them in the Cambridge-Ipswich area.

Clinical History

Of the nine cases, seven gave a history typical of acute gastro-enteritis; one had enteritis only, and the last patient, who was investigated for reasons not relevant to the present paper, was a symptomless excretor of the organism. The patients with gastro-enteritis complained of sudden onset of the disease with headache, abdominal pain, vomiting, and diarrhoea, and all showed some rise in temperature. The eighth case, a child, had similar symptoms except that she never vomited. Three patients were sufficiently ill to be admitted to hospital, one of them being described by her doctor as having symptoms suggestive of enteric fever. A fourth patient had been in hospital for six weeks with cardiac disease when he suddenly complained of diarrhoea; this case terminated fatally after a two-day illness, but it is impossible to say how far the *Salmonella* infection may have contributed to the fatal issue. The findings are summarized in Table I.

In all nine cases the organism (*Salm. birkenhead*, VI, VII; c ←→ 1,6) was isolated from the faeces. An attempt was made to determine whether any antibodies specific to the new *Salmonella* were present in the patient's serum on recovery. Sera

TABLE II

EXAMINATION OF SAMPLES OF BLOOD SERUM FROM PATIENTS

Case	Agglutination titre to *Salm. birkenhead*		
	0	H (c)	H (1,6)
J.B.	1:25	—	—
M.B.	1:25	1:320	1:640
A.G.	—	—	1:100
S.	1:50	—	1:50

All sera taken about four weeks after onset.

obtained from four cases gave the results shown in Table II. It will be seen that one case only, M.B., gave a high titre to the infecting organism. Such a finding is not uncommon in cases of infection

TABLE I

CLINICAL DATA AND DISTRIBUTION OF PATIENTS

Case	Age (years)	Symptoms	Duration of illness	Domicile	Date of onset
T.G.	46	G.E.	2 days (fatal)	London, Middx.	18.7.47
H.G.	Adult	—	—	Blunham, Beds.	15.8.47
J.B.	64	G.E.	27 days	Birkenhead, Lancs.	2.8.47
J.L.	2½	E.	7 days	Ipswich, Suffolk	18.8.47
E.A.	35	G.E.	15 days÷	Blunham, Beds.	11.8.47
M.B.	8	G.E.	7 days	Soham, Cambs.	25.8.47
A.G.	3	G.E.	9 days	Ipswich, Suffolk	29.8.47
S.	Adult	G.E.	7 days	Swindon, Wilts.	end of Aug. 47
L.	Adult	G.E.	7 days ÷	Newquay, Cornwall	25.8.47

G.E. = gastro-enteritis.   E. = enteritis.   — = no symptoms.   ÷ = patient not recovered when last seen.

with members of the *Salmonella* group, in which a rise in antibody titre is much more variable than in enteric infection.

Epidemiology

Since all the nine cases described occurred during a six-week period, it was thought that some article of diet, such as a new type of food, might be responsible for this infection. Home visits were paid early in October, 1947, to the five persons in the Cambridge-Suffolk area. Inquiries were made into any unusual food eaten by the patients, into the local sources of food, into visits by patients to other places, and into visitors to the household during the three weeks before onset. No lead was obtained to suggest that further investigation would determine the source of infection. In one instance (A.G.), there was another case of gastro-enteritis in the household which preceded the case described (A.G.) by thirty hours; this patient—a child—was not investigated.

Bacteriology

On MacConkey agar the Gram-negative motile bacillus isolated from the faeces of the cases described gave typical *Salmonella* colonies about 3 mm. in diameter, round, with slightly crenated edge and glistening surface. The biochemical reactions were as follows: glucose, maltose, mannitol, dulcitol, sorbitol, rhamnose, xylose, and trehalose were fermented with the production of acid and gas in twenty-four hours, and arabinose in three days. Lactose, saccharose, salicin, adonitol, dextrin, and inositol were not fermented within three weeks. Gelatin was not liquefied, indole was not produced, and urea was not broken down. The Voges-Proskauer test was negative, methyl red test positive, hydrogen sulphide was produced in lead acetate agar, and the citrate utilization test was positive.

All nine strains gave identical serological reactions in that they were agglutinated to titre by *Salm. oranienburg* "O" serum. Reciprocal absorption tests by a heterologous strain removed all the homologous agglutinins. Thus the somatic antigenic complex is VI, VII. Of the nine strains, five were agglutinated to titre by *Salm. paratyphi C* "H" (e) serum, two to titre by *Salm. anatum* "H" (1,6) serum, and two to titre by both these sera. On growing the five strains in "H" (e) phase in sloppy agar containing antiserum *Salm. paratyphi C* "H" (e) a second phase "H" (1,6) was isolated. Similarly on growing the two strains in "H" (1,6) phase in sloppy agar containing *Salm. newport* var. *puerto rico* "H" (1,2,3) serum,

the first phase "H" (e) was isolated. Reciprocal absorption of *Salm. paratyphi C* "H" (e) serum with the first phase of the new organism and a serum made against the new organism with *Salm. paratyphi C* "H" (e) completely removed all the homologous agglutinins. Similarly reciprocal absorption tests using *Salm. anatum* "H" (1,6) serum and an antiserum to the new strain proved these two phases to be identical. The flagellar antigens are therefore represented as e $\longleftrightarrow$ 1,6.

Discussion

The main interest in this new organism, *Salm. birkenhead* (VI, VII; c $\longleftrightarrow$ 1,6) lies in its close serological relationship to *Salm. paratyphi C* (VI, VII [Vi]; e $\longleftrightarrow$ 1,5), *Salm. cholerae-suis* (VI, VII; c $\longleftrightarrow$ 1,5) and *Salm. cholerae-suis* var. *kunzendorf* (VI, VII; e $\longleftrightarrow$ 1,5).

Salm. paratyphi C may contain the Vi antigen found in many strains of *Salm. typhi* and *Salm. ballerup*; this antigen was absent from all nine strains of *Salm. birkenhead*, so in this respect the somatic complex differed from many but not all strains of *Salm. paratyphi C*. *Salm. cholerae-suis* and the kunzendorf variety both have the same somatic complex as *Salm. birkenhead*. It is well known that the flagellar antigen e can be obtained from the kunzendorf variety of *Salm. cholerae-suis* (Gard, 1937; Bruner and Edwards, 1939); therefore serologically the only constant difference between *Salm. paratyphi C*, *Salm. cholerae-suis*, *Salm. cholerae-suis* var. *kunzendorf*, and *Salm. birkenhead* is that the second phase of the first three is 1,5, of the last 1,6.

The diagnostic biochemical reactions of *Salm. paratyphi C*, *Salm. cholerae-suis*, the kunzendorf variety, and *Salm. birkenhead* are given in Table III. From this it will be seen that the reactions of *Salm. paratyphi C* and *Salm. birkenhead* are very similar.

TABLE III

COMPARISON OF DIFFERENTIAL BIOCHEMICAL
REACTIONS

	Arabinose	Dulcitol	Rhamnose	Trehalose	H_2S
Salm. paratyphi C	$-+_1$	$+_1$	$+$ $_{1-2}$	$+$ $_{2-3}$	$+$
Salm. cholerae-suis	$-$	$\mp$	$+_1$	$-$	$-$
Salm. cholerae-suis var. *kunzendorf* ..	$-$	$\mp$	$+_1$	$-$	$+$
Salm. birkenhead	$+_3$	$+_1$	$+_1$	$+_1$	$+$

$-$ negative. $+_1$ acid and gas in 1 day. $\mp$ negative or slight positive.

In spite of the close relationship between the four organisms discussed, the clinical picture is very different. *Salm. paratyphi C* infection normally produces a serious illness, usually with continued fever. Complications of a septic type are not uncommon sequelae (Topley and Wilson, 1946), and two fatal cases of meningitis due to this organism have been described (Beattie and others, 1946).

Salm. cholerae-suis and the kunzendorf variety also cause serious disease. Harvey (1937) states that in sporadic cases in America the case-fatality rate in patients under 25 years of age is 19 per cent, in patients over 25 years of age 58 per cent. In his review of the literature describing epidemics due to this organism, he finds only five fatal cases among the 1,425 mentioned. Patients not uncommonly have sequelae such as pulmonary, bone, and joint infections, and endocarditis. He also describes four fatal cases of meningitis. In this country there have been few cases of this infection; twelve were studied by Scott (Reports 1923 to 1939). During this period there were in addition four examples of generalized infection (Nabarro and others, 1929, two cases; Boycott and McNee, 1936, one case; Guthrie, 1941, one case). In this laboratory seven strains were studied between 1940 and 1947; two of these caused fatal septicaemia (Schwabacher and others, 1943): of the remaining five, three were isolated from blood cultures, two from faeces: all five patients recovered.

Salm. birkenhead caused clinical disease typical of ordinary food-poisoning, and though some cases were serious enough to be admitted to hospital, none could be described as being dangerously ill, except the one fatal case where death was attributed to primary cardiac disease. After a six-month interval no patient is known to have suffered from sequelae. From this review it seems probable that though *Salm. paratyphi C*, *Salm. cholerae-suis*, the kunzendorf variety, and *Salm. birkenhead* are very similar in their biochemical and serological reactions, yet the type of clinical disease produced in man may be very different.

Summary

1. A new *Salmonella* type, *Salm. birkenhead*, with the antigenic structure VI, VII ; c $\longleftrightarrow$ 1,6 has been described. The Vi antigen was not detected in any of the strains.

2. This organism was isolated from the faeces of eight cases of food-poisoning and one symptomless carrier.

3. The difference in antigenic structure and clinical disease caused by *Salm. birkenhead* and the closely related organisms *Salm. paratyphi C*, *Salm. cholerae-suis* and *Salm. cholerae-suis* var. *kunzendorf*, are discussed.

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PENICILLIN BLOOD LEVELS AFTER INJECTION OF SOLID AND SEMI-FLUID OILY SUSPENSIONS OF PENICILLIN

BY

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The object of injecting an oily suspension instead of an aqueous solution of penicillin is to secure delayed absorption from the depot in the muscle and to achieve a prolongation of effective penicillin blood levels from a single injection. Many vehicles have been recommended, but so far a suspension of penicillin in beeswax and arachis oil, as used by Romansky and Rittman (1944), has proved the most satisfactory. In a previous communication (J. Ungar, 1946), I have shown that the degree of prolongation of penicillin action depends, apart from individual variations in the absorptive capacity of experimental animals or patients, to a large extent on the consistency of the material (which is determined by the viscosity of the oil and by the amount of beeswax) and on the amount of penicillin in the suspension.

In a number of experiments performed since then we have repeatedly confirmed that the delayed absorption of penicillin is governed by the consistency of the final preparation; see, for example, Table I.

We have tried arachis oil, " alcoholized " arachis oil,* ethyl oleate, and cocoa butter and found that arachis oil is the most suitable medium for the suspension. We tried altering the concentration of beeswax from the original recommended by Romansky. We used suspensions of penicillin in oil containing 1, 2, 3, 4, and 6 per cent of beeswax, and we found that the amount of beeswax present in the final preparation is an essential factor in determining the absorption of penicillin (Tables I and II). Lowering the amount of beeswax to between 1 and 2 per cent results in a more fluid product with the obvious advantage of easier manipulation, but the blood levels after injection of these products are less satisfactory.

The technique of injecting the different preparations was constant, and possible variations of volume and temperature of the injected suspensions were rigorously controlled in all experiments.

* A mixture of ethyl esters of the total fatty acids of arachis oil.

The penicillin-oil-beeswax (P.O.B.) prepared according to the original method of Romansky has disadvantages from the point of view of administration, for it is solid at normal temperatures and has to be heated to about 50° C. before it can be injected. Anyone who has to administer the preparation knows how cumbersome it is to fill the syringe with the heated P.O.B. and inject it quickly before it solidifies. For this reason methods have been suggested to increase the fluidity of the preparation, not by decreasing the content of beeswax, but by one of the special means used for preparation of colloids.

Milled Penicillin and Beeswax

A product of this kind is a suspension of penicillin in oil and beeswax made by passing the mixture through a special colloid mill. This milled preparation, containing 4.5 per cent of beeswax, is semi-solid at room temperature; when heated to body temperature it can be easily drawn into a syringe and injected. Table I and the Figure show blood levels in rabbits injected with the solid
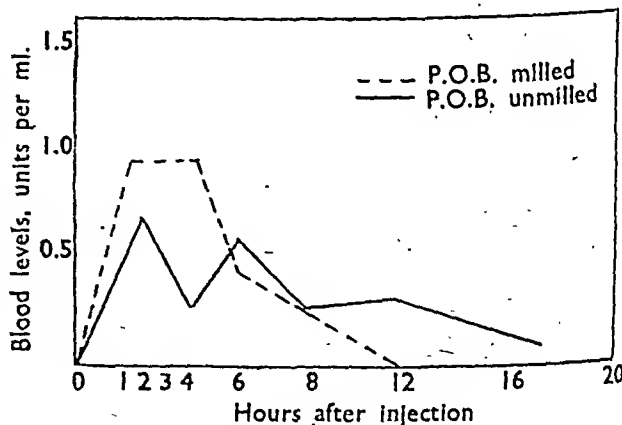
FIG.—Comparison of penicillin-oil-beeswax, milled and usual unmilled at room temperature (142,000 units per ml.) (20,000 units injected in 900 g. rabbits).

TABLE I

BLOOD LEVELS IN RABBITS AFTER INTRAMUSCULAR INJECTION OF 50,000 UNITS OF PENICILLIN IN OIL-BEESWAX UNMILLED SUSPENSIONS

Penicillin	% Beeswax	Rabbit no.	Hours after injection									
			2	4	6	8	10	12	14	16	18	20
Crystalline sodium	Nil	1	2.0	0	0	0	0	0	0	0	0	0
		2	2.0	0	0	0	0	0	0	0	0	0
	1	3	2.0	0	0	0	0	0	0	0	0	0
		4	2.0	0.03	0	0	0	0	0	0	0	0
	2	5	2.0	2.0	1.0	0.5	0.3	0.6	0	0	0	0
		6	2.0	2.0	1.0	0.4	0.1	0	0	0	0	0
	4	7	1.5	2.0	2.0	1.0	0.25	0.06	0.12	0.1	0.03	0
		8	2.0	2.0	1.0	0.5	0.3	0.12	0.12	0.03	0	0
Amorphous calcium	Nil	9	—	—	—	—	0	0	0	0	0	—
		10	—	—	—	—	0	0	0	0	0	—
	1	11	—	—	—	—	0	0	0	0	0	—
		12	—	—	—	—	0	0	0	0	0	—
	2	13	—	—	—	—	0.03	0.03	0	0	0	—
		14	—	—	—	—	0.12	0.03	0	0	0	—
	4	15	—	—	—	—	0.25	0.25	0.03	0	0	—
		16	—	—	—	—	0.25	0.1	0.1	0.06	0	—

and semi-solid preparations, both containing the same amount and type of penicillin and 4.5 per cent of beeswax.

We see from Table III that with this preparation we sacrifice part of the prolonged activity. Instead of a period of from sixteen to twenty hours during which penicillin is circulating in the blood after an injection of solid P.O.B., as reported previously (Ungar, 1946), we get, after the injection of a milled suspension, detectable blood levels up to a period of twelve to fourteen hours, which is satisfactory when treatment is given twice in twenty-four hours. In Table IV are given the blood levels of patients injected intramuscularly with 1 mL of a milled suspension of penicillin containing 300,000 units, from which it can be seen that a level of up to 0.1 unit per ml. is maintained during a period up to six hours and a level of 0.06 unit per ml. up to eleven hours.

Discussion

Penicillin suspensions in oil and beeswax prepared by simple mixing of the components are at ordinary temperatures solid and difficult to

TABLE II

BLOOD LEVELS IN RABBITS AFTER INTRAMUSCULAR INJECTION OF 30,000 UNITS IN 0.1 ML. OF UNMILLED SUSPENSION

Sample	Penicillin	% Beeswax	Rabbit no.	Hours after injection						
				1	3	5	7	8	12	24
EPC 10/54	Crystalline sodium	1	17	4.0	0.03	0	0	0	—	—
			18	4.0	0.06	0	0	0	—	—
EPC 10/55		4	19	4.0	1.0	0.5	0.2	0.06	0	0
			20	4.0	1.0	0.25	0.03	0.03	0	0
EPC 10/56	Amorphous calcium	1	21	4.0	0.5	0.1	0.03	0	0	0
			22	4.0	0.12	0.03	0	0	0	0
EPC 10/57		4	23	4.0	1.5	0.75	0.5	0.4	0.06	0
			24	4.0	1.5	0.75	0.25	0.1	0	0

TABLE III

BLOOD LEVELS IN RABBITS AFTER INTRAMUSCULAR INJECTION OF 20,000 UNITS OF PENICILLIN-OIL-BEESWAX
(MILLED AND UNMILLED)

Sample	Rabbit no.	Hours after injection						
		1	2	4	6	8	12	24
PC 1/16 (milled) ..	25	0.25	1.0	2.0	0.5	0.5	0.03	Nil
	26	−0.5	1.5	0.5	0.5	0.25	Nil	Nil
	27	0.25	1.0	1.0	0.25	0.125	Nil	Nil
	Mean	0.33	1.16	1.16	0.42	0.3	0.01	Nil
PC 2/31 (unmilled)	28	0.5	0.5	0.25	0.7	0.25	0.125	Nil
	29	0.125	0.5	0.125	0.5	0.25	0.5	0.03
	30	0.125	1.0	0.5	0.5	0.4	0.5	Nil
	Mean	0.25	0.67	0.3	0.57	0.3	0.375	0.01

administer. It is possible to achieve fluidity at body temperature either by reducing the amount of beeswax from the original 4.5 per cent to 1 per cent or by submitting the suspension to a special milling process. The first alternative results in a marked drop in the time of circulation of penicillin in the blood ; the second method results in a partial reduction of the circulation time. For the milled preparation it is still essential to add 4.5 per cent of beeswax to the oil suspension ; otherwise the period of penicillin circulation in the blood drops. In our experiments we have observed that suspensions containing crystalline sodium penicillin or amorphous calcium penicillin give similar blood levels. Further, we have so far been unable to detect any effect of particle size of amorphous calcium penicillin, when administered as P.O.B., on blood levels, and a slight improvement in one experiment with crystalline sodium penicillin is of doubtful significance.

In using the milled penicillin beeswax suspension we make a compromise—to achieve an increased fluidity of the product we sacrifice to some extent the prolonged period of blood levels following injection of a solid penicillin suspension. The results of our tests confirm our earlier finding of an inverse relation between the fluidity of penicillin suspensions and the time during which the penicillin circulates in the blood.

One additional point should be emphasized when comparing the blood levels of penicillin after injections of solid and milled P.O.B. The solid suspension usually gives blood levels up to a period of about eighteen hours, whereas the milled P.O.B. maintains blood levels over a shorter period, though the initial levels in the first one to three hours are higher than after injection of the solid P.O.B. This initial higher blood level (Table III and Figure) compensates for the reduced penicillin circulation in the blood and makes the milled suspension second only in value to aqueous solutions when higher bactericidal blood levels are required—blood levels essential to suppress microorganisms less susceptible to penicillin, such as occasionally occur in cases of septicaemia or chronic localized infections.

Summary

1. The effect of penicillin in oil-beeswax suspension depends on the consistency of the material

TABLE IV

BLOOD LEVELS IN PATIENTS INJECTED WITH 1 ML. OF MILLED PENICILLIN SUSPENSION 300,000 UNITS PER ML.

Patient	Hours after injection										
	1	2	3	4	5	6	11	15	16	18	20
1	0.25	0.25	0.25	0.125	0.125	0.1	0.06	0.06	0.06	0.03	Nil
2	2	1	1	0.5	0.5	—	0.06	Not tested further			
3	2	2	1	1	1.5	0.2	—				

and the amount of penicillin present. Amorphous calcium penicillin and crystalline sodium penicillin give equally effective products.

2. The amount of beeswax is one of the essential factors in delaying the absorption of penicillin.

3. The " milled " penicillin beeswax suspension is characterized by its ease of administration and gives blood levels midway between those following injections of the solid penicillin-oil-beeswax or of aqueous solutions of penicillin.

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Addendum

At the time of reading the proof of this communication, which had been reported at the January, 1948, meeting of the Association of Clinical Pathologists, I came across a paper by Dowling and others on " liquid " versus " solid " penicillin in oil and wax (*J. Amer. med. Ass.*, 135, 567). This report gave the results of treatment of patients with fluid beeswax suspension. Their investigation seems to indicate that fluid suspensions may have similar absorbtion-delaying effect as solid suspensions if the former are prepared from penicillin particles of 50 μ size or larger.

EMBOLISM AFTER PENICILLIN-OIL-BEESWAX

BY

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(RECEIVED FOR PUBLICATION, MAY 19, 1948)

The occurrence of fat embolism after injury to bone has been described and the literature adequately reviewed by Robb-Smith (1941) and by Rowlands and Wakeley (1941). Similar findings occur after the accidental intravenous introduction of oily substances. Grossmann (1946) described the *x*-ray appearances of two cases of pulmonary oil embolism following salpingography. The first patient had no symptoms for two days ; the second patient began to cough very soon after the injection of the oil. The *x*-ray films in the latter patient were taken within five minutes, when it was seen that the main filling of the pulmonary arterial tree occurred in the lower lobes. Many observers have stressed the delayed appearance of symptoms which occur after fat embolism. Harris and others (1939) suggested that this is due to hydrolysis of fat particles with the release of free fatty acids. Animal experiments that were carried out suggested that severe reactions may occur after an oil-beeswax mixture, not only because the mixture blocks large branches of the pulmonary artery, but also because of the severe inflammatory reaction elicited by beeswax.

When a patient dies·the recognition of a fat or oil embolism is not easy, because special methods have to be used to show the presence of these substances in the sections. In patients who recover the clinical picture is not clearly defined or easily recognized because the diagnosis has not been considered, especially where the symptoms are delayed. The development of pulmonary and cerebral signs and symptoms with petechial haemorrhages in the skin should all be looked for. Fat globules in the sputum and/or urine confirm the diagnosis. In many cases no abnormal signs are present and the symptoms are minimal.

I can find only one previous published account of oil embolism following penicillin in oil-wax therapy. Bondy and others (1947) describe a patient who had a severe reaction which showed maximal radiological changes on the second and third day after injection. The immediate effects were slight, consisting only of a penicillin-like taste in the mouth and a few coughs.

Two cases that occurred over two years ago had symptoms corresponding to a third described below, in that these symptoms were immediate and minimal, consisting of a tight feeling in the chest and a cough immediately after receiving an injection of penicillin in oil-wax.

Case Report

A married woman, aged 30, had been admitted for streptomycin therapy for persisting ulcerative colitis. While in the ward she had complained of chronic bronchitis, and by the time she had finished the streptomycin therapy there was no doubt that she had developed true asthmatic attacks. While awaiting discharge from hospital she was given an injection of 600,000 units in 2 ml. of calcium penicillin. The penicillin was suspended in peanut oil with 4.8 per cent (w/v) beeswax B.P. The injection was given by a nurse who, on being questioned, could not be certain that the usual precautions of making sure that the needle was not in a vein had been carried out. Immediately after the injection the patient began to cough, said she felt sick, asked for a bed-pan, and then said she was going to faint. She was put back to bed and when seen five minutes later she said she was feeling quite well. She was surprised the injection had made her feel faint, as previously she had had many injections of either penicillin or streptomycin without ill effect. When she was examined there were no abnormal physical signs, other than those due to generalized bronchitis which had been noted previously. The bronchitis was productive of a little sputum, about 20 ml. of which was collected during the following day: this amount represented the expectoration from the time of waking until before the midday meal. Unfortunately no precautions were taken to make sure that this was not contaminated with any oil or fat from a food source. A further specimen of sputum was collected three days later and a control specimen of sputum taken from another

chronic bronchitic patient. Sudan III was used as an oil stain. Only in the specimen collected the first day after the penicillin injection and only in certain parts of the field could many oil globules be seen ; all these were extracellular, and most of them were very minute—about 5 μ in diameter—though a few of the large ones were 50 μ in size. Examination of a slide with the stained sputum on it, and a control of the unstained and also stained penicillin in oil-wax, showed that in no case was there rotation of the plane of polarized light. It is therefore suggested that the oil in the sputum had as its source the penicillin oil-wax injection given into the buttock.

Summary

A case is described in which a mild immediate reaction occurred after an injection of penicillin in oil-wax. It would appear that this was due to oil embolism.

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TECHNICAL METHODS

ANOTHER WAY OF APPLYING IMMERSION OIL

BY

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In a recent short paper published by Pijper (1947) an improved method of applying immersion oil to slides is described. It seems odd that laboratories should still employ the archaic method of applying the oil from a bottle by a rod, when not even a garage would dream of employing this method. I have tried many shapes of bottles and types of rods—made from glass, cane, or spring steel—and all of them were equally messy, wasteful, irritating, and inefficient ; if the bottle is too full a drop falls on the bench before the microscope is reached, and if the bottle is nearly empty insufficient oil adheres to the rod and two or three applications are necessary before the correct amount is obtained, and usually air bubbles are included. After reading Pijper's article I wondered if anyone had ever tried an oil-can.

The ideal oil-can for immersion oil must be flat-bottomed so as to stand upright on the bench, and must have a long, tapering stem and a narrow opening—all features being present in the original Singer oil-can.* An alternative oil-can called the Alton Valve Spout is nearly as good and is obtainable at any wholesale motor supplies distributors at a cost of 2s. 6d. ; this has an additional advantage of a screw valve on the stem which can be closed by half a turn, and is useful when the oil-can is being carried in a microscope case, as it does not leak when lying on
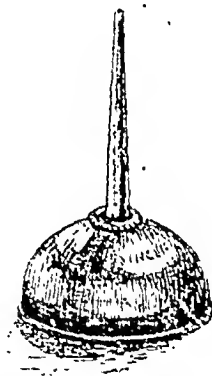
its side ; a valve is unnecessary, however, when the oil-can is standing on the bench. The amount of oil being applied is kept completely under control by pressing the bottom, and if this is done carefully there is no excess to run down the side of the stem and so soil the microscopist's fingers, the microscope, and the bench, wasting the immersion oil.

* Shortly available again from the Singer Sewing Machine Co., of Singer, Clydebank, Dumbartonshire, or agents, for 5d.

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AN EMERGENCY BOTTLE FOR DRIED PLASMA

BY

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Dried plasma is usually kept in evacuated, rubber-stoppered bottles. Reconstitution calls for a series of additional gadgets as the water has to be transferred from its bottle to the plasma bottle, and two rubber tubings have to be inserted in order to give access to the air and to carry the dissolved plasma. Furthermore, if the vacuum in the plasma bottle has decayed, the water has to be pumped over with a blowing ball. All these manipulations are not always easily performed in the field.

An attempt has therefore been made to design an emergency bottle for quickly reconstituting dried plasma. The underlying principle is that of the fire-extinguisher in which the contents are mixed after an ampoule has been crushed.

The Emergency Bottle

The emergency bottle (see Figure) consists of a cylindrically shaped outer vessel (1) whose lower end
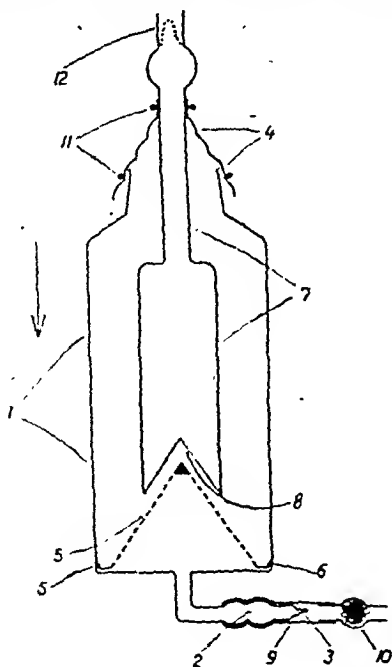
forms an olive (2), drawn out to a point and sealed (3). The wide upper mouth of this vessel forms a brim which engages a bellow-shaped rubber-connexion (4). On the bottom of the vessel rests a cone of stainless steel (5) with reinforced tip. This cone is a sieve with holes of about $1\frac{1}{2}$ mm. diameter resembling an inverted filter-cone. Its rim is flattened and is kept towards the bottom of the outer vessel (1) by four protrusions of the glass (6). The inner vessel (7) is a big ampoule with a stout neck which passes the rubber-connexion (4). The bottom (8) of the ampoule is drawn in and thinned. The neck of the ampoule is dilated near its top after the rubber-connexion (4) has been pushed over. This bellow-shaped rubber-connexion is cheaply available as rubber push-on connector for gas or water tubing; for larger bottles small rubber showers that may be attached directly to taps are a satisfactory alternative if the perforated plate is removed. A short piece of thick (pressure) rubber tubing (9), pushed over the olive, protects the drawn-out tip (3), and leads to a glass bulb (10). This bulb (10) is stuffed with 28- to 32-gauge bandage and acts as a filter. The free end of the bulb is connected to a needle protected against contamination (not shown in the drawing). A sinfered glass pipeline filter would probably be better than gauze bandage, but this could not be tried*; when assembling the apparatus, distilled water is filled into the outer vessel (1) and all parts are sterilized in the autoclave. The connector (4) is then secured to both vessels by circular wires (11). The distance between the bottom of the ampoule (8) and the steel cone (5) is determined by the flexibility of the rubber piece (4) so that accidental movements of the ampoule should not engage the steel cone. The ampoule (7) is dried by connecting to a pump, and dried plasma is then filled into the ampoule. The bulb in the neck of the ampoule is plugged with sterile cotton wool, evacuated, and sealed at (12) (dotted line).

When needed, the inner vessel (7) is pushed into the outer (1), in the direction indicated by the arrow. As soon as the bottom of the ampoule (8) is crushed, water is drawn in and mixes with the dry plasma. Shaking aids dissolution of the plasma. Then the tip of the ampoule (12) is opened to give access to the air, and flow of the plasma is started by bending the rubber tubing (9) whereby the tip (3) is being broken. The vessels can be re-used.

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* Messrs. Baird and Tatlock, London, recommend their K.301 H.3 or K.301 H.4 filter tube as a suitable sintered filter.

ESTIMATION OF STREPTOMYCIN AND PENICILLIN IN BLOOD

BY

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The method here described, which we have been using for some time in routine work, is a modification of the micro-method of May and others (1947), in which dilutions of the patient's serum are added to phenol-red-serum water on waxed slides, and incubated in capillary tubes.

The dilutions of patient's serum and their addition to the medium are performed on a waxed glass plate which is incubated directly, without filling the mixtures into capillary tubes and fixing to plasticine-covered slides. In this way the amount of manipulation is reduced and an appreciable saving of time is achieved, especially when several sera are being titrated at the same time. Moreover the colour change at the end point is even more obvious than in capillaries and there is no danger of upsetting the tests by rough handling..

Details of Method

The medium used is 1 per cent glucose in a mixture of serum 1 part, distilled water 4 parts, sterilized by steaming (May and others, 1947). We find it best to make up batches of this medium without indicator. A saturated solution of phenol red in distilled water is put up separately in small amounts, each sufficient for one batch of medium, and similarly sterilized. At the time of the test the indicator is added in the proportion of about 0.05 ml. to 1 ml. serum water. We have found that if the indicator is added to the medium some days before use, fading occurs and the colour changes after incubation are not so distinct. The medium is inoculated as in the original method (0.01 ml. of 24-hour broth culture of the test organism to 1 ml. medium).

The capillary pipettes used to measure unit volumes have tapered, slightly angled free ends; as in the original method, they are graduated to contain 0.025 ml.

For making and incubating the dilutions, waxed rectangular slabs of glass, in which are drilled a series of depressions, are used. The slabs are cut from plate glass, about 7 mm. thick, and measure approxi-

mately 7.5×4.5 cm. In one surface of each slab are drilled ten circular depressions 8 mm. in diameter and about 2 mm. deep at the centre. Each plate is placed in a 3½-in. Petri dish and sterilized in hot air. When cool it is grasped with sterile forceps and quickly dipped in melted sterile paraffin wax at about 60° C., briefly drained, and replaced in its dish.

Serial dilutions of the patient's serum and of a suitable standard solution of antibiotic in normal serum are made in equal volumes of sterile saline in the depressions (with the last as a control). To each is added an equal volume of inoculated medium. A quantity of water or of moistened filter paper is placed in the Petri dish, which is incubated, lid upwards, at 37° C. During incubation the dish should rest on wood or other heat-insulating material to avoid moisture condensation on the lid.

For streptomycin estimations we use klebsiella 41 as test organism and inactivate the patient's serum. For penicillin, the Oxford staphylococcus is used. As in the original method, it is advisable to aim at having the end point within the first five or six dilutions in order to minimize the cumulative error of the wash.

After incubation, the last dilution showing complete inhibition is taken as the end point. The strength of antibiotic is calculated by comparison with the standard. The drops of fluid retain their convex surfaces in the waxed depressions, thus rendering colour differences more striking; there is no tendency for them to overflow, even with quite rough handling (Plate IX).

Numerous titrations have been done in parallel with the capillary tube method, without significant differences in results.

Recently we have found (Goslings, 1947) an account of an essentially similar method for penicillin, using haemolytic streptococcus, with blood as the indicator.

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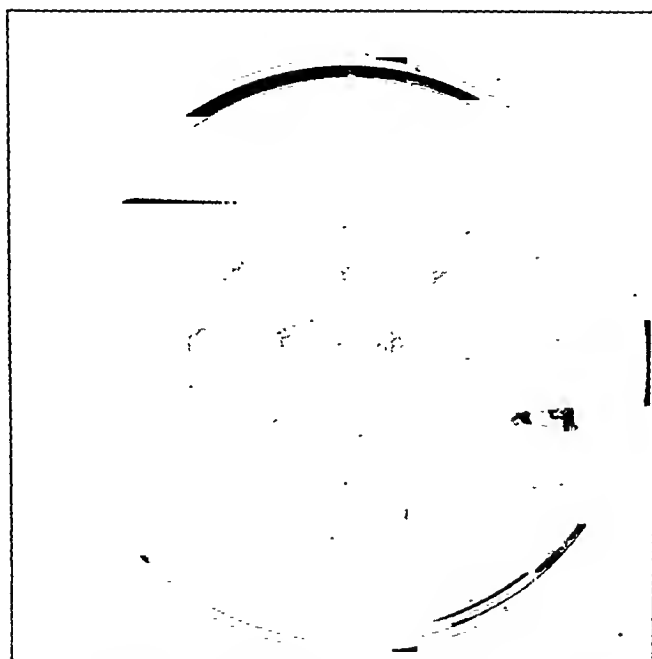
PLATE IX.—Waxed depression plate after incubation.
End point at dilution 1/64.

REVIEWS

Il Principio Antianemico-Pernicioso. By G. Astaldi and M. Baldini. Pavia: Il Farmaco, Monograph. January, 1948. Pp. 234, figs. 54. Price 1,200 lire.

This monograph on the pernicious anaemia factor by two members of Ferrata's school of haematology appears at a moment when the discovery of folic acid and the production of new and powerful liver concentrates have created fresh interest in and raised new questions about the origin and treatment of the macrocytic anaemias. The authors have set out to give an interim review of this rapidly changing subject, and have produced a clear and up-to-date summary of its theoretical and practical aspects.

The book is divided into two parts: the first deals with the nature of the liver principle and of the extrinsic and intrinsic factors, the properties of the various commercial and experimental liver extracts, desiccated stomach preparations, and the pteroylglutamic acid group. In a very full review of the older preparations covering both the English and continental literature, the only important omission is that of crude " proteolysed " liver. Recent work on folic acid, its conjugates, and thyamine is adequately described. The second part covers the methods of assaying the activity of therapeutic preparations both from the theoretical and practical aspects, and includes much of the author's original work. After a critical account of the common therapeutic tests, based on the reticulocyte and erythrocyte responses, they present a review of many other suggested methods of assay, all of which they reject as either non-specific or as yet insufficiently controlled. There are in fact no rapid or large-scale assay methods, and this explains most of the difficulties in research on new preparations. The author's own solution of the problem is based on studies of bone marrow both *in vivo* and *in vitro* and consists in a statistical analysis of the diminution in size, under treatment, of the red blood cell precursors ; but, as it involves multiple marrow punctures and a laborious technique, it is doubtful if this attractive suggestion will be widely adopted in practice.

Defects in an otherwise excellent book are the careless quotation of references and the absence of an index. In spite of these, it can be recommended to anyone in search of a full and balanced account of present-day knowledge of the nature and application of the substances used in the treatment of pernicious anaemia.

F. W. GUNZ.

Major Endocrine Disorders. By S. Leonard Simpson. Geoffrey Cumberlege, Oxford University Press. Second edition, 1948. Pp. 552+xxii. Price 42s.

The new edition of this book gives a good picture of the recent development of endocrinology. This speciality is growing fast, and it will be increasingly difficult for the general physician to keep abreast of its progress. The way the book is written will help him in this task. Each endocrine organ is treated separately ; after a short review of its physiology the clinical disorders connected with its dysfunction are dealt with. These are often accompanied by typical case histories and by excellent photographs. The pituitary gland and the gonads are given more space than the other endocrine organs ; in the latter some disorders are treated which cannot be strictly termed major disorders, but this is probably unavoidable. The chapter on the pancreas and diabetes mellitus is comparatively short. Biochemical methods used in the diagnosis of the disorders described are not given, and the biochemical data are sometimes scanty, but this is compensated by the introduction of ample references. On the whole, the author has successfully avoided cramming his book with too much detail ; it will be a welcome help to the student and to the clinician.

H. HERXHEIMER.

Nutrition and Hormones. By L. T. Samuels. American Lecture Series No. 11. Blackwell Scientific Publications, Oxford. 1948. Pp. 48. Price 8s. 6d.

This is a series of short essays on the mutual interaction of hormones and nutritional factors. It aims at correlating the gross metabolic changes in endocrine diseases on the one hand with the effect of various types of malnutrition on the endocrine glands on the other. Since these materials make up the greater part of modern endocrinology the result is a rather brief account of the relationships of the various hormones which is perhaps too incomplete to be of value to the average pathologist or clinician. For example, the effect of iodine deficiency on the thyroid gland is dismissed in six lines and that of thiouracil in four. The book is attractively bound and the well chosen bibliography of 108 recent references is a valuable introduction to the literature.

N. F. MACLAGAN.

OBITUARY

JAMES McINTOSH

On April 5 this year Pathology suffered a great loss in the death of James McIntosh, Professor of Pathology in the University of London and Director of the Bland-Sutton Institute of Pathology at the Middlesex Hospital since 1920. He was a man of wide interests, with an international reputation as an authority in several spheres of pathological research.

He was educated at Robert Gordon's College and at the University of Aberdeen, where he qualified in 1905 and was awarded the M.D. with highest honours in 1908. After spending two years with Levaditi at the Pasteur Institute during his tenure of the Alexander Anderson Scholarship, he returned to Aberdeen in 1908 as Carnegie Research Scholar. In 1910 he became a Grocers Research Scholar at the London Hospital, where he worked in association with William Bulloch, Paul Fildes, and H. M. Turnbull until 1920 when, at the age of 37, he was appointed Professor of Pathology and Director of the Bland-Sutton Institute.

His first outstanding contribution to medical science was his pioneer work with Fildes on the perfection of the Wassermann reaction and the application of Ehrlich's new remedy, salvarsan, in the treatment of syphilis. This investigation was a natural sequel to his researches with Levaditi at the Pasteur Institute on *Treponema pallidum* and other spirochaetes. It was there, also in collaboration with Levaditi, that he performed some of the earliest experiments on the development of resistance to arsenical drugs in micro-organisms. He was always interested in the problem of drug resistance, and he returned to it almost forty years later to make fundamental contributions to our knowledge of the development of resistance in bacteria to the sulphonamides, penicillin, and the acridines. His exceptional grasp of the fundamentals of chemotherapy was again shown in the views he expressed with Whitby in 1939 on the mechanism of sulphonamide action, which were later confirmed and brought to fruition by the discovery of the role of *p*-aminobenzoic acid by Fildes and Woods, who were then housed in his Institute. He also played an important part in the clinical application of the new antibacterial drugs, particularly with respect to the bacteriological control and methods of administration—as will be recalled by members of the Association of Clinical Pathologists who heard his address on subacute bacterial endocarditis at the winter meeting two years ago.

JAMES McINTOSH

An achievement which alone would have established his reputation in medical research was his classification of the anaerobic bacteria associated with war wounds. He took up this subject during the 1914–18 war, and it is not too much to say that his work made order out of the chaos then existing because of the inadequacy of the methods in current use for the isolation of these organisms. In this difficult subject he displayed great technical dexterity in the new methods he introduced and, with Fildes, he evolved the anaerobic jar that bears their names and is the

indispensable apparatus for the critical study of anaerobes. His report to the Medical Research Council on anaerobic infections is a classic used by all workers in the most exacting discipline in all bacteriology. His services in this field were again called upon in the 1939–45 war, when he was appointed a member of the War Wounds Committee of the Medical Research Council, who profited not only from his wide experience but also from the investigations he conducted on many urgent problems. Indeed one would not hesitate to say that the high standard of the work done on anaerobic infections during the last war was largely due to his life-long interest in the anaerobic bacteria and to the inspiration he gave to many other workers.

Another sphere in which McIntosh gained distinction was the experimental study of virus diseases. He was first actively involved in this field at the London Hospital where, with H. M. Turnbull, he commenced a study of encephalomyelitis following vaccination, which he continued at the Bland-Sutton Institute. In the report on this subject to the Ministry of Health in 1925 McIntosh found himself so much at variance with his colleagues that he submitted a minority report expressing his conviction that vaccinia virus could not be excluded as a cause of post-vaccinial encephalomyelitis. It was not until many years later that his views on the generalization of vaccinia virus and the causation of post-vaccinial encephalitis became widely accepted. In later years he applied his experience in handling viruses to the experimental study of cancer. He made the important observation that tumours induced by tar in fowls could be transmitted by cell-free filtrates. Although other workers have brought forward indirect evidence in support of his findings, none has succeeded in repeating his demonstration of a virus agent in these induced tumours. He was firmly of the opinion that viruses played the dominant part in the aetiology of tumours, and, with the further inroads that are being made by virologists into the tumour problem, his work is now gaining increasing respect. In his handling of viruses his mechanical genius came to the fore—as it had done before in the design of the anaerobic jar—as exemplified by his adaptation of the spinning-top ultracentrifuge for the sedimentation of viruses and his modification of the Sharples centrifuge for the continuous centrifugalization of large quantities of virus suspensions.

McIntosh will also be remembered for the smooth-running organization he built in the Bland-Sutton Institute, which he administered from 1920. Here, besides carrying on his many scientific investigations, he was responsible for undergraduate teaching in pathology and also for the pathological services to the Middlesex Hospital. His outside activities in the service of pathology were many. He was a member of the Pathological Society of Great Britain and Ireland, for which he acted as treasurer for many years, a senior member of the Medical Research Club, a past-president and representative to the Library Committee of the Pathological Section of the Royal Society of Medicine, and an honorary member of the Association of Clinical Pathologists. He also took a deep interest in the welfare of his laboratory technicians and did great service for many years in the Pathological and Bacteriological Laboratory Assistants Association, now the Institute of Medical Laboratory Technology. He was also an examiner to the Universities of London, Cambridge, and Manchester, and for the Conjoint Board.

In the 1939–45 war he was pathologist to Sector V of the Emergency Medical Service and directed two laboratories in the Aylesbury district in addition to the Bland-Sutton Institute. As Chairman of the London Sector Pathologists Committee he also played his part in co-ordinating the pathological services of the London area. In addition to his work in the Sector and for the Medical Research Council, he also conducted an investigation of cases of encephalomyelitis on behalf of the Ministry of Health.

McIntosh was a bachelor of a rather retiring disposition, and to strangers and even to many acquaintances he was sometimes difficult to understand. His shyness and habit of self-effacement frequently gave the impression that he was somewhat brusque and disinterested. Those who knew him well, however, were aware that it cost him a great effort to show his displeasure and that when he did so some fundamental principle was involved. Misunderstanding also sometimes arose from his way of expressing himself, which led to apparent contradictions, largely because of his habit of letting his thought outstrip his speech. Even so, one was frequently startled by the rapidity with which he got to the core of a complicated subject and summed it up in a few words by a process which defied analysis.

He had many interests outside his profession, such as his series of high-powered cars, his golf, and his garden. He was a genial host and helped many in their private worries. There are many who will miss McIntosh, not only as an inspiring chief but also as a loyal friend.

F. R. SELBIE.

We regret to record the death of Dr. Gordon Wilkinson Goodhart; an obituary notice will appear in the next issue.

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ABSTRACTS

This section of the JOURNAL *is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this* JOURNAL *some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections : bacteriology ; biochemistry ; haematology ; and morbid anatomy and histology.*

BACTERIOLOGY

Improvement of the Diagnosis of Tuberculosis by Guinea-pig Inoculation. (Amélioration du bactério-diagnostic de la TBC par l'inoculation au cobaye.) BADOUX, V. (1947). *Schweiz. Z. Path. Bakt.*, 10, 470.

A review is made of technical details of guinea-pig inoculation in the diagnosis of tuberculosis, and a plea is entered for always basing a report on results in 2 guinea-pigs. The material should be kept, and used to inoculate additional guinea-pigs should the originally inoculated animals die prematurely. The author claims an increase of 4.76% positive results since he has used 2 animals for every test. R. Salm.

Detection of Mycobacterium tuberculosis by Means of Fluorescence Microscopy. BRIGGS, G. O. A., and JENNISON, M. H. (1947). *Tubercle, Lond.*, 28, 189.

The authors tested the value of the fluorescence method as used in a sanatorium of 210 beds admitting all types of pulmonary tuberculosis. A series of sputum smears was stained by the auramine-phenol technique (*Brit. J. Tuberc.*, 1946, 111, 98). After examination these films were then re-stained by a Ziehl-Neelsen technique described by Jennison (*Bull. Inst. Med. Lab. Techn.*, 1946, 6, 87). Of 500 smears thus examined 44.4% were positive with the fluorescence technique and 45.8% by the modified Ziehl-Neelsen method. Thus the modified Ziehl-Neelsen method yielded 1.4% more positive results with an expenditure of 6 seconds less time per smear, or an overall saving of 50 minutes.

Detection of Latent Genital Tuberculosis by Culture of Menstrual Discharge. HALBRECHT, I. (1947). *Lancet*, 2, 947.

Endometrial biopsies have shown that latent genital tuberculosis is commoner than hitherto suspected, occurring in at least 5% of cases of primary sterility. Endometrial biopsy, however, can give a positive result only when tuberculosis has reached that particular portion of the endometrium removed for scrutiny. The author undertook culture of menstrual blood obtained with a vaginal speculum from the fornix and the os on the first or second day of the menses, and repeated his examination at least thrice in each patient. Of 140 tests in 80 women with primary sterility, including 10 with proved endometrial tuberculosis and 2 others clinically suspected of genital tuberculosis, culture was positive nine times in 6 women. The author suggests that this is the only safe method of establishing the diagnosis where genital tuberculosis is clinically suspected.

Culture of menstrual blood was positive in only 2 of the 10 proved cases of endometrial tuberculosis.

J. A. Chalmers.

Alcohol as a Disinfectant Against the Tubercle Bacillus. SMITH, C. R. (1947). *Publ. Hlth Rep., Wash.*, 62, 1285.

The author reviews previous work on the efficiency of alcohol as a disinfectant, particularly against the tubercle bacillus. Water is essential to the disinfectant action of ethyl alcohol; the optimum strength appears to be 50 to 70%. The germicidal effectiveness of aliphatic alcohols increases with molecular weight as far as the amyl derivatives, and then decreases through octyl to undecyl alcohol. Normal propyl alcohol is the most effective.

Experimental data are given, and it is concluded that both ethyl and *iso*propyl alcohols are useful and practicable disinfectants against the tubercle bacillus and that they are specially suitable for skin disinfection. They are also suitable for thermometers and for surfaces, dishes, and handicraft articles which might be damaged by other methods of disinfection. Some plastics, painted and varnished surfaces, and some fabrics and dyes, may be damaged by alcohol, and in thoracoscopes and cystoscopes the lens systems may be held in position by alcohol-soluble cements and so may be liable to harm.

R. J. Lumsden.

Relative Numbers of Resistant Tubercle Bacilli in Sputa of Patients before and during Treatment with Streptomycin. PYLE, M. M. (1947). *Proc. Mayo Clin.*, 22, 465.

In 7 out of 8 cases of pulmonary tuberculosis a few moderately resistant organisms were present in cultures from sputum before the start of chemotherapy. During treatment with streptomycin the proportion of resistant bacilli in cultures recovered from sputa at weekly intervals increased, the degree of their resistance increased, and the predominantly sensitive reactions of the isolated strains, as judged by the ordinary methods of testing sensitivity, were replaced by predominantly resistant reactions. P. D'Arcy Hart.

Estimation of Streptomycin in the Blood and Cerebro-spinal Fluid. (Titrage de la streptomycine dans le sang et le liquide céphalo-rachidien.) CHABBERT, Y., and SUREAU, B. (1947). *Ann. Inst. Pasteur*, 73, 1142.

The test organism used is the Oxford strain of *Staphylococcus aureus* grown in glucose broth with phenol-sulphonphthalein at *p*H 8.4 as indicator. The organism

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is inhibited in this medium by 0.44 unit of streptomycin per ml. A series of control tubes containing various dilutions of streptomycin is read against dilutions of the fluid, serum, plasma, or cerebrospinal fluid in the medium, the presence of growth being shown by change in the indicator. The tubes showing complete inhibition and just failing to show inhibition are noted. The method is sensitive to 0.44 unit per ml., and can be applied to urine provided the urine is sterilized by passage through a Seitz filter. *G. M. Findlay.*

Further Observations on the Occurrence of Streptococci of Groups other than A in Human Infection. FOLEY, G. E. (1947). *New Engl. J. Med.,* 237, 809.

The grouping of strains of streptococci isolated from sources other than the upper respiratory tract was studied. Out of 118 cases investigated, 95 showed organisms of strains other than group A: 77 belonged to groups B, C and G, D, E, F, and K. The streptococci most frequently encountered in the study were those of group D, and these were usually associated with endocarditis, urinary tract infections, and intra-abdominal abscesses. Streptococci belonging to groups B, C and G, E, F, and K were met with in adult and childhood infections. The author believes that the incidence of streptococci of groups other than A cannot be properly ascertained if only colonies giving a beta reaction on blood agar are studied. *J. Smith.*

Staphylococcal Infection Due to Penicillin-resistant Strains. BARBER, M. (1947). *Brit. med. J.,* 2, 863.

In a series of examinations undertaken during April–November, 1946, 99 patients yielded 14 strains of *Staph. pyogenes* from infected lesions resistant to penicillin; during February–June, 1947, 38 out of 100 patients yielded resistant strains. It would appear that 10 from the last group also harboured sensitive strains at the beginning of treatment. The sensitive strains were found to belong to serological and bacteriophage types different from those of the resistant strains.

The author brings forward evidence to show that the penicillin-insensitivity is not due to resistance built up in the tissues after administration of penicillin, but that the strains are originally resistant to the action of penicillin. They are penicillinase producers and flourish in a lesion only after the sensitive strain has been overcome by penicillin.

In determining the penicillin-sensitivity of these penicillinase-producing organisms the size of the inoculum was found to be important. The ditch-plate method was particularly valuable, for unless the size of the inoculum was carefully controlled some of the other methods for the determination of sensitivity might yield misleading results with penicillinase-producing bacteria. *H. J. Bensted.*

Treatment of Diphtheria Carriers with Penicillin. (La profilassi penicillinica nei portatori di bacilli difterici.) TORRICELLI, C., and MONTAGNA, A. (1947). *Lattante,* 18, 474.

A series of 414 children, mostly infants, was examined for diphtheria organisms. Of the 22 strains recovered, 1 was a toxic *Corynebacterium diphtheriae,* 12 were non-toxic *C. diphtheriae,* and 9 were "pseudo-diphtheria" bacilli. The gravis organism was isolated from a child who had a sero-sanguineous nasal discharge and was probably suffering from diphtheritic rhinitis. The other organisms came from the nasopharynx of healthy carriers and from pus in 3 cases of chronic purulent otitis media.

All 22 children were treated by the nasal or aural instillation of penicillin solution, 1,000 units per ml., 4 to 6 times a day for about 10 days, and the organisms disappeared. *E. G. Sita-Lumsden.*

The Suppressive and Schizonticidal Value of Paludrine (100 mg.) in Vivax Malaria. WOODRUFF, A. W. (1947). *Trans. R. Soc. trop. Med. Hyg.,* 41, 263.

Fifteen patients suffering from benign tertian malaria were treated with a single 100-mg. tablet of "paludrine." The results compared unfavourably with those obtained in a similar series treated by quinine for 3 days followed by mepacrine. It is suggested that this dose of paludrine is sub-optimal in an acute attack. Twenty patients, after an acute attack of benign tertian malaria treated by paludrine, were given 100 mg. paludrine weekly for 6 months. No toxic manifestations were noted. During the period of paludrine administration one short-term relapse and three possible clinical attacks occurred. During the 6 months following the period of paludrine administration there was 1 proved long-term and 1 possible clinical relapse. Thus, in the dosage used, it seems that paludrine does not invariably prevent long-term relapses. *J. L. Markson.*

Contribution to the Serological Diagnosis of Amoebiasis. (Contributo allo studio della sierodiagnosi nell' amebiasi.) RITA, G. (1947). *Riv. Parassit.,* 8, 113.

The author carried out complement-fixation tests with an antigen made by Craig and Scott's technique on 63 patients with amoebic infection and on numerous controls: 14 had acute dysentery, 2 amoebic hepatitis, and 47 intestinal amoebiasis. In 1 patient with hepatitis the entamoebae were not found, but they were seen in all the others. Fifty-eight gave a positive result. Of the 5 negative cases, 2 had acute dysentery and 3 had mild intestinal forms. Persistence of the complement-fixation reaction indicates that the patient is not free from his amoebae and relapse is likely.

Meningitis Leptospirosa. BUZZARD, E. M., and WYLIE, J. A. H. (1947). *Lancet,* 2, 417.

This paper records 5 cases aged 9 to 23 years in which Weil's disease was characterized solely by mild benign meningitis with certain particular features, especially suffusion of conjunctivae. Three had recently been river bathing and the other two had occupational risks. The cerebrospinal fluid had a slightly raised pressure and a cell count of 50 to 300 per c.mm. with varying proportions of granulocytes and lymphocytes. There was slight proteinuria, but no cells or casts in the urine. No patient was jaundiced. In all cases the agglutination titre rose to 1 in 1,000 or higher at the end of the second week. All recovered completely without specific treatment of any kind.

Meningitis due to Candida (Monilia) Albicans with Recovery. ZIMMERMAN, S. L., FRUTCHEY, L., and GIBBS, J. H. (1947). *J. Amer. med. Ass.,* 135, 145.

This is believed to be the first recorded case of recovery from meningitis proved to be due to *Candida albicans,* the 3 previously recorded cases having all ended fatally. A man aged 28 had a week's history of increasing malaise, headache, fever, vomiting, and drowsiness alternating with delirium. White patches were present on the palate, posterior pharynx, and back of the tongue. He had neck rigidity, a positive Kernig sign, absent deep reflexes, and retention of urine. His cerebrospinal fluid contained 37 lymphocytes per c.mm. and 186 mg.

protein and 585 mg. chlorides per 100 ml. A direct smear of the fluid stained with methylene blue showed large, oval, budding yeast-like fungi, which could be cultured and subcultured with production of mycelia. Serum agglutination against *C. albicans* was positive at a 1 in 40 dilution.

The condition responded to an 8-day course of streptomycin, and he eventually made a complete recovery. *A. H. Stewart-Wallace.*

Observations on the Epidemiology of Poliomyelitis in Glasgow. ANDERSON, T. (1947). *Glasg. med. J.*, 28, 328.

The epidemiology of poliomyelitis is discussed in the light of recent experience of the disease in Glasgow. It seems probable that the final number of cases in Glasgow in 1947 will not be far short of three-quarters of the total number of cases notified in the past 27 years. This high prevalence justifies a discussion of the possibilities: (a) that a new virus may have appeared; (b) that the virus normally present endemically may have acquired an increased power of attack; or (c) that the human herd may have become more susceptible because of some unknown environmental change. The author inclines to the view that person-to-person infection, chiefly from healthy carriers and abortive cases, is the most important means of spread. *W. H. Bradley.*

The Isolation of a Filterable Agent Pathogenic for Mice from a Case of Reiter's Disease. DUNHAM, J., ROCK, J., and BELT, E. (1947). *J. Urol.*, 58, 212.

Conjunctivitis was produced in mice by intraperitoneal injections of filtered allantoic fluid previously infected with material from the urethra and conjunctiva of a 23-year-old white man suffering from Reiter's disease with conjunctivitis, urethritis, and arthritis uninfluenced by sulphathiazole, penicillin, or neoarsphenamine.

The Harris Slide Test. A Microflocculation Test for Syphilis with Cardiolipin Antigen. VOGELSANG, T. M. (1947). *Brit. J. vener. Dis.*, 23, 109.

Cardiolipin was used for the Venereal Disease Research Laboratory slide test by Harris *et al.*, who reported highly satisfactory results with a low incidence of false positive reactions (*J. vener. Dis. Inform.*, 27, 169), as did Kline (*Arch. Derm. Syph.*, Chicago, 1947, 55, 514) when contrasting this test with the other flocculation procedures.

The present author employed what he christens the "Harris test" on 5,556 sera, and compared the results with the standard Kahn and Meinicke and with Bordet-Wassermann tests. Of 777 known syphilitic sera there was agreement between results of the Harris and Bordet-Wassermann in 81.85%. Of the remaining 141 sera 100 gave a positive Harris reaction and 99 a positive Wassermann, while negative results were obtained in 41 and 42 respectively. Of 4,779 non-syphilitic sera there was agreement in 98.72%, although, of the 61 remaining, 43 gave a negative Harris reaction and only 10 a negative Bordet-Wassermann. Thus the two tests were about equally sensitive, the Harris being the more specific. The Harris test showed similar sensitivity and somewhat greater specificity than the standard Kahn: even if less sensitive than the Meinicke, it was considerably more specific. *R. R. Willcox.*

BIOCHEMISTRY

A Survey of the Accuracy of Chemical Analyses in Clinical Laboratories. BELK, W. P., and SUNDERMAN, F. W. (1947). *Amer. J. clin. Path.*, 17, 853.

In 1946 the Committee on Laboratories of the Medical Society of Pennsylvania carried out a survey to check the accuracy of findings in clinical laboratories. Some 59 pathologists in hospitals of all types expressed their willingness to analyse standard samples sent to them. Each received 12 samples, previously analysed, of aqueous solutions of glucose, chloride, urea, and calcium in concentrations commonly found in the blood, together with a sample of serum for protein estimation, and samples of whole blood for haemoglobin determination. Their findings were entered on plain printed cards, which were returned unsigned. The data were arranged in frequency distributions, and histograms are reproduced. The referee selected reasonable limits of error permissible for each analysis, and grouped results as "satisfactory" and "unsatisfactory" according as they fell within or outside these limits. The authors found a surprising degree of unreliability; measurements were below any reasonable standard of accuracy. Unsatisfactory results outnumbered the satisfactory ones, and gross errors were not uncommon. Of 51 haemoglobin determinations, only 17 were satisfactory (limits 9.8 ± 0.3 g. per 100 ml.); of 52 glucose determinations (limits 60 ± 10 mg. per 100 ml.) 19 were unsatisfactory; and of 44 serum albumin determinations only 9 were satisfactory. Roughly one-third of the calcium estimations were satisfactory, and two-thirds of the sodium chloride estimations.

A questionary on these unsatisfactory results brought replies from 106 pathologists. The majority attributed the inaccuracy to poor training and shortage of technicians, while 64 thought that there was lack of understanding between pathologist and staff.

S. S. B. Gilder.

(This very important survey should be read and the figures and tables studied in the original. A similar survey carried out in this country would certainly reveal the poor correlation existing among laboratories, even when they are using similar methods.—Ed.)

Methionine Excretion. Effect of Diet and Methionine Ingestion in Normal Subjects. TIDWELL, H. C., SLESINSKI, F. A., and TREADWELL, C. R. (1947). *Proc. Soc. exp. Biol., N.Y.*, 66, 482.

The daily urinary excretion of methionine in 80 male medical students on a normal control diet containing approximately 2 g. methionine per day, as determined by the method of Albanese (*Bull. Johns Hopk. Hosp.*, 1944, 75, 175), averaged 318 ± 9 mg. (range 199 to 518 mg.) or about 4.5 mg. per kilo body weight. The methionine excretion during 6-hour and 24-hour periods after the ingestion of 1 or 1.5 g. methionine was determined in 26 subjects given food and 11 subjects given none. The increase in methionine excretion was approximately 9 and 15% of the amino-acid supplement.

Three groups of 12 subjects were then placed on a diet with a low and a higher protein content and a high fat content respectively. The calculated daily methionine content of the diets was 1.34, 3.33, and 2.23 g. After 4 days no change in the methionine excretion was observed in the subjects on the diets with low or higher protein content, but there was a decrease in the methionine excretion in the subjects on a high fat diet, and a similar decreased excretion was observed when the latter

subjects were given a 1.5 g. supplement of methionine. Nine subjects who fasted for 3 days showed a diminished urinary excretion of methionine. It is suggested that an increased lipotropic requirement might account for diminished excretion of methionine in subjects on a high fat diet or after a 3-day fast. *C. C. N. Vass.*

Plasma *dl*-Methionine Levels Following Intravenous Administration in Humans. HARPER, H. A., KINSELL, L. W., and BARTON, H. C. (1947). *Science*, **106**, 319.

After a 12-hour fast a blood sample was withdrawn for determination of plasma methionine, and 50 ml. of 3% solution of *dl*-methionine was then given intravenously to 11 subjects. Methionine determinations were then carried out on the plasma and urine at intervals during 3 hours. Following a sharp rise in plasma methionine 15 minutes after injection the concentra ion slowly fell, but in no case did it reach the pre-injection level within the 3 hours of observation. There were considerable individual variations in rate of disappearance and in urinary excretion. The excretion was most rapid during the first 15 to 30 minutes when the blood levels were highest, but the amount excreted at any time was quite low when compared with the quantity injected. *D. T. Barry.*

Use of Methionine and Vitamin Supplements in Treatment of Hepatic Disease. Clinical and Laboratory Observations. CAYER, D. (1947). *Arch. intern. Med.*, **80**, 644.

The author divides 18 cases into three groups: (1) 4 of acute infective hepatitis (2) 6 of chronic hepatitis without ascites, and (3) 8 of chronic hepatitis with ascites and signs of collateral venous circulation. The patients were given a daily oral dose of 3 to 6 g. of methionine and therapeutic doses of the vitamin-B complex, in addition to a diet of 3,500 calories made up of 120 to 140 g. of protein, 130 to 150 g. of fat, and 350 to 400 g. of carbohydrate given in multiple small feeds. Those in hospital were given 500 to 1,000 ml. of 10% dextrose solution intravenously daily. In group 1 the course of the disease was not appreciably altered. In group 2 considerable improvement, as judged by clinical observation and by liver-function tests, was noted in all cases within 7 days. In group 3, 7 out of 8 patients showed some clinical improvement, and 3 were well and carrying on full-time work on an average 13 months after treatment. The author comments on the well-known poor prognosis in this disease and quotes for comparison larger series reported by others and treated on different lines. *J. B. Mitchell.*

The Thymol Turbidity Test as a Measure of Liver Disease. With Special Reference to Comparison of the Turbidity at 18 Hours with that at 30 Minutes ("18 Hour Turbidity Ratio"). SHAY, H., BERK, J. E., and SIPLET, H. (1947). *Gastroenterology*, **9**, 641.

The Thymol Turbidity Test and Impaired Liver Function. MANN, F. D., SNELL, A. M., and BUTT, H. R. (1947). *Gastroenterology*, **9**, 651.

Studies of Responses of Certain Hepatic Tests in Diseases of the Liver and Biliary Tract. I. Serum Cephalin Cholesterol Flocculation, Thymol Turbidity, Thymol Flocculation and Colloidal Gold Responses. NEEFE, J. R., BAHNSON, E. R., and REINHOLD, J. G. (1947). *Gastroenterology*, **9**, 656.

These three interesting papers deal with the now muchused turbidity or flocculation tests of hepatic function.

Shay and others consider particularly the thymolturbidity test, but contrast it clinically with the cephalin-cholesterol and colloidal gold tests. They suggest a new modification of the thymol reaction—namely, a reading at 18 hours as well as at 30 minutes—and find that the "18-hour turbidity ratio" remains abnormal in infective hepatitis longer than do the other flocculation tests.

Mann and others have also used the thymol-turbidity test, and agree with previous workers that it is usually positive in infective hepatitis and negative in biliary obstruction. In other forms of hepatic disease the results are very variable. The authors' comment on the possible explanation of the flocculation is worth reading. They emphasize that the bromsulphthalein retention test is still the best indicator of injury to the liver when jaundice is absent.

Neefe and others found that the cephalin-cholesterol test provided evidence of hepatic disorder more often than did the other tests, but was for this reason of less value in distinguishing between intrahepatic forms of jaundice and extrahepatic biliary obstruction. They suggest that the thymol and gold tests are likely to have a similar mechanism, since their results run parallel and may differ considerably from those with cephalincholesterol. They conclude that a combination of all three tests is often useful in diagnosis, but if two only are chosen they should be the cephalin-cholesterol and thymol reactions.

(These papers support other workers' opinions of the value of these three tests of disturbed hepatic function, and will encourage research into their mechanism.) *J. W. McNee.*

The Colloidal Scarlet Red Test Applied to Cerebro-spinal Fluid. (La reacción del rojo escarlata coloidal aplicada al liquido cefalorraquídeo.) TRIGUEROS, E. A., and REINLEIN, J. M. A. (1947). *Rev. clin. esp.*, **27**, 182.

Gray (*Arch. intern. Med.*, 1940, **65**, 523) suggested the use of the colloidal gold curve for testing hepatic function. Maizels proposed the substitution of the scarlet-red test instead of the colloidal gold test; this in turn led the authors of this paper to think that the scarlet-red test might be useful for replacing Lange's colloidal curve in examinations of the cerebrospinal fluids. They have examined 96 cases, and find that Lange curves and the curves obtained with scarlet red are for practical purposes identical. As the scarlet-red test is simpler and cheaper to carry out, the authors recommend it instead of Lange's gold test. *F. K. Kessell.*

Studies on the Mechanism of the Fanconi Syndrome. STOWERS, J. M., and DENT, C. E. (1947). *Quart. J. Med.*, **16**, 275.

The authors record a case in a man of 35 who had severe osteomalacia in the absence of any dietary deficiency of vitamin D, mild diabetes with renal glycosuria, amino-aciduria (without excessive cystine or methionine excretion), hypophosphataemia, and moderately impaired renal function; the liver showed focal centrilobular necrosis with nodular hyperplasia and a carcinomatous change (malignant hepatoma). Details of the investigations, including special metabolic studies of the amino-acid and sulphur metabolism and the ionic equilibrium of the serum and urine, are recorded. No significant change in the metabolism of sulphur-containing amino-acids was found. It is suggested that the excessive excretion of glucose and amino-acids is

dependent on the lack of adequate phosphate for their effective phosphorylation; thus their tubular reabsorption is impaired; besides a low renal threshold for phosphates, hyperphosphaturia may result from a chronic mild acidosis. *Henry Cohen.*

The Influence of Stilbamidine upon Kidney Function, Liver Function, and Peripheral Blood in Multiple Myeloma. ARAI, H., and SNAPPER, I. (1947). *N.Y. St. J. Med.*, 47, 1867.

The authors discuss the toxic effects of stilbamidine, and report their findings in the treatment of 26 patients suffering from multiple myeloma. The kidney and liver functions were studied during and after stilbamidine therapy. In 24 patients there were no significant changes suggestive of impairment of kidney or liver function during or after stilbamidine therapy. Two patients who showed evidence of renal impairment during treatment were proved to have serious involvement of the kidney with myeloma. The authors conclude that although there is no conclusive evidence of renal or hepatic damage due to treatment with stilbamidine the influence on the kidney function of patients with myeloma must be carefully watched, especially in those with Bence-Jones proteinuria. *G. E. Hesketh.*

Diagnosis of Adrenal Tumours. A New Chemical Test. PATTERSON, J. (1947). *Lancet*, 2, 580.

The differential diagnosis of adrenal tumour from adrenal hyperplasia is not aided by the estimation of the total urinary 17-ketosteroids, since they are increased in both conditions. One member of the group, however, dehydroisoandrosterone, is increased in adrenocortical tumours in females. A new colour reaction is described for this substance. The reaction was positive in 3 cases of adrenal tumour with a very high daily ketosteroid output (215,335, and 1,980 mg.), negative in 6 cases of prepuberal virilism (output 24 to 81 mg.), and negative in 8 cases of secondary virilism (output 21 to 30 mg.). *H. Herxheimer.*

The Renal Regulation of Acid-base Balance in Man. I. The Nature of the Mechanism for Acidifying the Urine. PITTS, R. F., LOTSPEICH, W. D., SCHIESS, W. A., and AYER, J. L. (1948). *J. clin. Invest.*, 27, 48.

The rate of excretion of titratable acid in the presence of acidosis with a concomitant infusion of large quantities of neutral sodium phosphate (pH 7.4) or creatinine was investigated. Eight experiments were performed on 4 healthy adult males. Acidosis was induced by the ingestion of 20 g. of ammonium chloride on the day preceding the observations. "Arterialized" venous blood was obtained from an arm vein for determinations of pH and CO_2 content. Glomerular filtration rate was determined by thiosulphate clearance; the plasma thiosulphate concentrations were maintained between 30 and 40 mg. per 100 ml. Simultaneous measurements of the rates of filtration, reabsorption, and excretion of monobasic and dibasic phosphate and carbonic acid showed that the quantity of titratable acid excreted was much greater than that which was filtered off in the glomerulus. When the plasma phosphate concentration reached 6 to 7 mM (millimols) per litre the hypothetical filtration-reabsorption mechanisms for phosphate and bicarbonate together could account for only one-third of the titratable acid excreted. Acid must have been added to the urine by the renal tubule. The rate of ammonia excretion averaged 0.066 mEq (milliequiva-

lents) per minute which, though much increased over normal, was not high compared with that observed in diabetic acidosis. The ratio of ammonia to titratable acid, normally between 1 and 2.5, fell with the infusion of phosphate to 0.16 solely because of the increase in rate of excretion of titratable acid. In three experiments on 2 subjects the pH of the urine ranged between 4.48 and 4.6. Three experiments on 3 different subjects were made in which creatinine was substituted for phosphate. Again the carbonic-acid filtration could account for only 35 to 56% and the phosphate reabsorption for only 5.4 to 8.7% of the excreted acid. The mechanism for acid excretion in the human kidney is qualitatively similar and, judged by these experiments, quantitatively greater than that described in the dog kidney (Pitts and Alexander, *Amer. J. Physiol.*, 1945, 144, 239). *C. C. N. Vass.*

The Renal Regulation of Acid-base Balance in Man. II. Factors Affecting the Excretion of Titratable Acid by the Normal Human Subject. SCHIESS, W. A., AYER, J. L., LOTSPEICH, W. D., and PITTS, R. F. (1948). *J. clin. Invest.*, 27, 57.

This paper records a study on one subject (R.F.P.) but the results obtained were confirmed on other normal adult males. With the subject in a state of moderate 24-hour acidosis (plasma bicarbonate concentration 14.4 mM (millimols) per litre, plasma pH 7.37) the amount of phosphate in millimols excreted per minute during infusion of neutral phosphate bore a direct relation to the amount of titratable acid in milliequivalents excreted per minute. The results are in accord with the view that the urine is acidified by the exchange of hydrogen ions formed within the tubular cells for ions of fixed base in the tubular urine. *C. C. N. Vass.*

A New Clearance Substance in Renal Function Tests. (Eine neue Clearance-Substanz zur Nierenfunktionsprüfung.) FUHRMANN, G., and SCHUBERT, H. (1947). *Z. ges. inn. Med.*, 2, 451.

The use of inulin and of creatinine clearance substances in testing renal function is unsatisfactory. A new agent, triticin, has been employed; it is also a fructose-yielding polysaccharide but of low molecular weight and soluble in water (25%). It may be injected rapidly and never causes shock. It is excreted completely in the urine in about 4 hours, none being retained by the tissues. Hydrolysis and the Selivanoff test, employed quantitatively with photometric measurement, proved satisfactory for estimation of triticin. The normal clearance values as well as the abnormal are approximately those obtained with inulin. *D. T. Barry.*

The Titratable Acidity, pH, Ammonia and Phosphates in the Urines of Very Young Infants. McCANCE, R. A., and VON FINCK, M. A. (1947). *Arch. Dis. Childh.*, 22, 200.

For some time after birth the kidney function is still immature, and it has been shown that the glomerular filtration rate, and the urea, sodium, and chloride clearances are low in newborn babies. This paper extends these observations by furnishing data on the pH, the titratable acidity, the ammonia coefficients, and the phosphate in the urine of newborn babies born in occupied Germany. Comparisons are made with specimens obtained from healthy Britains and Germans.

The data are based on the examination of urine from 72 infants, 36 adults, and 10 diabetics. All the babies were breast-fed and no additional water was given. The

following results were obtained: (1) The average pH tended to fall slightly after birth with an increase as milk flow was established. The pH did not differ from the average in urine from healthy adults. (2) The titratable acidity varied with the concentration of the urine and fell as the pH rose. (3) Ammonia coefficients were unaffected by the age of the baby but on the average were higher than in normal adults. (4) The ratio of the ammonia to the acidity was higher in infants than in adults. (5) Urine of newborn infants contained very little phosphate. This might lead to an incapacity to deal with an acidosis, should one occur. (6) Urine passed *in utero* was very dilute but differed in no other way from that passed in the first few days of life.

A. G. Watkins.

Response of New-born Children to Hypertonic Solutions of Sodium Chloride and of Urea. DEAN, R. F. A., and McCANCE, R. A. (1947). *Nature, Lond.*, **160**, 904.

The authors examined the diuresis following injection of hypertonic saline and urea in infants suffering from meningomyelocele, and compared the findings with those in normal adults. While a diuresis occurred in both infants and adults, this was much less marked on a basis of surface area in infants, while after saline the osmotic pressure of the urine behaved differently in the two groups, falling in adults and rising in infants. Excretion of administered salt was much slower in infants. Similar results were obtained with urea, though the discrepancies between age groups were less marked and the diuresis less.

Alex Comfort.

Influence of the Diet on Urea Clearance. (Kostens Indflydelse paa Urinstof-clearance.) BANG, H. O., and NIELSEN, A. L. (1947). *Nord. Med.*, **36**, 2376.

The authors report the effects of alternate high-protein and low-protein diets on urea clearance in 5 nurses and 5 patients with healthy kidneys. The good diet was the full Danish hospital diet with 100 g. of meat added daily; the poor diet contained 25 to 30 g. of protein daily and had a caloric value of about 2,000. Subjects were given the diets for from 6 days to over 3 weeks and were in nitrogen balance; the nurses lost some weight when on the poor diet.

Urea clearance was estimated during two 1-hour or three $\frac{1}{2}$-hour periods with an indwelling catheter. Diuresis was maintained to give maximal clearances. In all there were 25 estimations after a good diet and 15 after a poor one; with insignificant exceptions, individuals on the poor diets showed a fall in clearance down to as low as 44% of the previous values; the average fall was to about 70%. On return to the good diet clearance rose again. Other features of the poor-diet period were lowered urinary output (average 700 against 950 ml. a day), low plasma-urea levels (average 19 against 28 mg. per 100 ml.) and a low nitrogen output (average 4.1 against 10.7 g. a day). *A. M. M. Wilson.*

Examination of Diodrast Clearance and Tubular Excretory Capacity in Man by Means of Two Single Injections of Diodrast (Umbradil). (In English.) JOSEPHSON, B. (1947). *Acta med. scand.*, **128**, 515.

A method of testing renal function is described based on changes of the plasma and urine concentration of "diodrast" after one intraglutaeal and one intravenous injection, combined with a clearance test of creatinine and inulin.

Nitrogen and Fluid Balance in Treatment of Acute Uremia by Peritoneal Lavage. Analysis of Peritoneal Washings for Protein, Nonprotein Nitrogen and Phosphorus. BASSETT, S. H., BROWN, H. R., KEUTMANN, E. H., HOLLER, J., ALSTINE, H. E. V., MOCEJUNAS, O., and SCHANTZ, H. (1947). *Arch. intern. Med.*, **80**, 616.

A woman aged 21, who in the course of progressive subacute glomerulo-nephritis became uraemic and practically anuric, was treated during the last 21 days of her life by peritoneal lavage. Altogether 257 g. of nitrogen was removed in the washing (140 g. of non-protein nitrogen and 117 g. of protein nitrogen), in addition to 92 g. in the gastric contents obtained by suction and 5 g. in the urine. At first substantial reduction in the non-protein nitrogen in the blood and inorganic phosphorus in the serum were obtained, but as the patient lost oedema fluid the values finally exceeded the prelavage figures. *L. H. Worth.*

The Urinary Excretion of Amino Acids by a Cystinuric Subject. YEH, H. L., FRANKL, W., DUNN, M. S., PARKER, P., HUGHES, B., and GYÖRGY, P. (1947). *Amer. J. med. Sci.*, **214**, 507.

In 7 normal subjects the amino-acid excretion showed a remarkably uniform pattern, regardless of age differences. In a cystinuric child there were marked changes from the normal in the excretion of some amino-acids, both in absolute and relative amounts. Ingestion of methionine increased the urinary excretion of cystine in the cystinuric patient. It appears that in the normal subject arginine, cystine, and histidine, and in the cystinuric patient arginine, cystine, and lysine are present mainly in the unconjugated state, since the values for these amino-acids were essentially the same before and after hydrolysis of the urine. *L. H. Worth.*

Chemical Studies in Children with the Nephrotic Syndrome. GOTTFRIED, S. P., STEINMAN, J. F., and KRAMER, B. (1947). *Amer. J. Dis. Child.*, **74**, 283.

For 2 years the authors studied 10 children between 16 months and 5 years old; 2 of the children died during this period. Extensive examinations were made of the blood chemistry and hepatic function. An increase or decrease of oedema seemed dependent on the serum protein and serum albumin being below or above 4 g. and 1.5 g. per 100 ml. respectively. Therapeutically, concentrated plasma and various forms of protein hydrolysate failed to produce any constant effect upon the total protein and serum-albumin levels. Some impairment of hepatic function was noticeable in all cases, and a defective production of protein is postulated. Low calcium values in serum were consistently present, and slight generalized osteoporosis was visible in radiographs. *W. G. Wyllie.*

The Addis Count in the Prognosis of Acute Nephritis in Childhood. GILES, M. D. (1947). *Arch. Dis. Childh.*, **22**, 232.

The Addis count was used as a guide to prognosis in acute haemorrhagic nephritis. A red cell count of 600,000 per c.mm. or under in an 18-hour specimen of urine was considered to be normal. The result of the Addis count in 218 cases of acute haemorrhagic nephritis was compared with results of renal function tests, including estimations of non-protein nitrogen, serum proteins, and urea concentration and clearance tests. If the Addis count returns to normal and remains so it may be assumed that the renal lesion is healed and that a relapse is unlikely. *A. G. Watkins.*

Comparative Absorption, Excretion, and Storage of Oily and Aqueous Preparations of Vitamin A. LEWIS, J. M., BODANSKY, O., BIRMINGHAM, J., and COHLAN, S. Q.— (1947). *J. Pediat.*, 31, 496.

Previous observations by these authors indicated that aqueous solutions of vitamin A are much better absorbed by premature infants than oily preparations, and the present investigation was undertaken "to ascertain whether the same phenomenon obtained for full-term infants, children, and adults." It was found that the average maximum blood level of vitamin A was five times higher after the aqueous product than after the oily solution. That this was due almost entirely to better absorption was shown by the fact that an average of 38% was wasted in the stools after ingestion of the oily product, whereas only 7% of the vitamin-A intake was lost in the stools when the aqueous solution was given. Similar studies on an 8-year-old boy with cystic fibrosis of the pancreas suggest that it should be possible to eliminate vitamin-A deficiency as a complication of this disease. It should also be possible to improve the absorption and effectiveness of vitamin D in the prevention and treatment of rickets. *M. Baber.*

Thiamine, Riboflavin, Nicotinic Acid, Pantothenic Acid and Biotin in the Urine of Newborn Infants. HAMIL, B. M., CORYELL, N., RODERUCK, C., KAUCHER, M., MOYER, E. Z., HARRIS, M. E., and WILLIAMS, H. H. (1947). *Amer. J. Dis. Child.*, 74, 434.

Estimations of the daily urinary excretion of the vitamins of the B complex was undertaken in the newborn as a preliminary to assessing the nutritional status of the babies as regards these substances. Maximal average concentrations of thiamine and of pantothenic acid were found in urine excreted on the third day of life. For riboflavin the maximal average value was found on the first day, and for nicotinic acid and biotin on the second. The concentration of all vitamins in the urine decreased greatly after the first few days, very low levels being reached by the fifth day post partum in spite of abundant intake of breast milk. This large excretion of vitamins presumably indicates a high foetal storage. *M. Baber.*

The Effect of Dietary Restriction of B-complex Vitamins and Protein on the Excretion of Creatinine by Human Subjects. FRIEDEMANN, T. E., KINNEY, V. M., BERRYMAN, G. H., HENDERSON, C. R., and YOUMANS, J. B. (1948). *J. Nutrit.*, 35, 117.

Seven men, aged 22 to 27 years, initially physically fit, were given 3 different diets and physical exercises over a period of 50 weeks, during which the variations in creatinine excretion and their relation to changes in weight and physical performance were studied. The results support the theory of early physiologists that there is a "constancy" in the relation between the creatinine excretion and the physical and nutritional state of the individual.

Vitamin C in the Blood and Urine of the Newborn and in the Cord and Maternal Blood. HAMIL, B. M., MUNKS, B., MOYER, E. Z., KAUCHER, M., and WILLIAMS, H. H. (1947). *Amer. J. Dis. Child.*, 74, 417.

The plasma concentration of reduced ascorbic acid was estimated in samples of cord blood, capillary blood from 24 male breast-fed infants, and venous blood from their mothers, and compared with the amounts of vitamin C excreted in the infant's urine during the first week of life. The results showed wide variations, but cord blood contained by far the highest concentration of vitamin C, and the average value for the babies' blood, though only about half that for cord blood, was higher than for mothers'. Concentrations of vitamin C in urine were high during the first 2 days of life, but if the babies were not supplied with vitamin C urinary excretion dropped rapidly to low levels or disappeared as the blood level of the vitamin decreased. *M. Baber.*

Changes in Serum Calcium and Inorganic Blood Phosphorus after Treatment with Massive Doses of Vitamin D₂ in Some Cases of Tuberculosis in Children. (Variazioni del calcio sierico e del fosforo inorganico ematico dopo urterapia D₂ in alcuni casi di T.B.C. infantile.) RAGAZZINI, F. (1947). *Riv. Clin. pediat.*, 45, 473.

The behaviour of blood calcium and inorganic phosphorus levels was studied in 14 children with tuberculosis and 1 normal control; all were given massive doses of vitamin D_2 (calciferol), with and without intravenous calcium.

Cases were divided into 3 groups of 5. Patients in the first 5 were given 600,000 units intramuscularly and the serum calcium was estimated 1½, 3, 6, 24, 27, 33, 48, 51, 57, 72, and 96 hours later. There was a maximum rise of calcium late on the second or early on the third day to 0.8–1.9 mg. per ml. above the "resting" value; this maximum was maintained for 12 to 24 hours, and thereafter the level slowly fell, but in no case did it reach the former "resting" figure within 4 days. There was a small initial drop in blood calcium at 1½ hours. The second group was investigated over a period of 2 weeks, with daily serum calcium estimations for the first 7 days, and then on alternate days. A maximum rise on the third day was confirmed, and some cases showed a second but lesser peak about the sixth to eighth day. The blood calcium returned to its former level during the second week, in all cases by the fifteenth day. In the third group were 4 children with some form of tuberculosis, and 1 control. Phosphorus levels were found to parallel closely those of calcium in the serum, but the rise was more persistent. The daily intravenous injection of 10 ml. of 10% calcium gluconate produced greatly increased serum calcium, and the rise was more prolonged but there was no further effect on the phosphorus level. If calcium was given alone without vitamin D_2; there was no significant change in either the calcium or the phosphorus level, but if the vitamin was then administered there was an immediate and marked rise in calcium. Calcium given a week after the injection of calciferol had no effect in raising the calcium level in the blood. The normal control showed no change in phosphorus levels in spite of increase in serum calcium. The author recommends simultaneous administration of calcium and vitamin D_2, as calcium is then better utilized and the initial hypocalcaemic effect is obviated. *F. G. Sita-Lumsden.*

Utilization of Parenteral Protein Hydrolysate in the Normal. BARBORKA, C. J., CARROLL, W. W., and HEPLER, O. E. (1947). *Gastroenterology*, 9, 579.

The preparation used in this study was "aminosolfibrin," a partial acid hydrolysate of fibrin, 5% w/v, with 5% dextrose. About one-third of the amino-acids was in free form, and the remainder approximately in the tripeptide state. There were no toxic reactions, provided the solution was administered at a rate not

exceeding 80 drops a minute. Ten healthy 75-kg. medical students were chosen for the experiment. The administration of hydrolysate was preceded by a control period when the students were subjected to a protein intake varying between 75 and 10 g. daily. On this dietary a slight to definite negative nitrogen balance existed in all the subjects. A significant positive balance was obtained by the daily administration of 2 litres of the hydrolysate in all except those subjects whose diet contained only 10 g. of protein, these required 3 litres. The administration of larger doses was found to be unnecessary and wasteful. The positive balance was obtained only while the hydrolysate was being administered, and was rapidly lost during the ensuing 24 hours.

During administration there was no elevation of the non-protein nitrogen content of the blood and no significant alteration in the serum proteins.

J. B. Hannah.

The Effect of Dietary Fat upon Gastric Evacuation in Normal Subjects. ANNEGERS, J. H., and IVY, A. C. (1947). *Amer. J. Physiol.*, 150, 461.

The authors, investigating the statement that fat delays gastric emptying, administered standard meals containing varying proportions of fat, and studied the emptying of the stomach by radiography. They conclude that while there is no difference in effect between saturated and unsaturated fats, statistically significant delay occurs in most subjects receiving a high-fat meal.

Alex. Comfort.

Anomalies of Intestinal Absorption of Fat. II. The Haematology of Idiopathic Steatorrhoea. COOKE, W. T., FRAZER, A. C., PEENEY, A. L. P., SAMMONS, H. G., and THOMPSON, M. D. (1948). *Quart. J. Med.*, 27, 9.

It is interesting to have fresh confirmation that the pale faeces of steatorrhoea contain normal or increased amounts of bile pigments. The authors are inclined to attribute any increased pigment excretion to increased haemolysis rather than to a failure of absorption.

L. P. R. Fourman.

The Chylomicron Count in Normal Subjects and Patients with Sprue. FOURMAN, L. P. R. (1948). *Trans. R. Soc. trop. Med. Hyg.*, 41, 537.

The difficulties involved in counting chylomicrons are discussed, but the general conclusion is that counts of the fatty particles in serum do give some indication of the amount of absorbed fat in the serum at the time of the count. The chylomicron count does not, however, bear a close relation either to total fat absorption or to any individual fraction, though of these it is most nearly related to the neutral fat fraction. In patients with sprue depressed chylomicron curves were common, although the degree of depression was little related to the severity of the fat absorption defect as judged by results of fat-balance studies; moreover, treatment with liver improved the chylomicron count without greatly affecting the steatorrhoea. The author's deduction is that the proportion of absorbed fat which contributes to the chylomicron count is small. On the other hand, Elkes Frazer, and Stewart have shown that "chylomicron fat" represents an appreciable portion of the serum fats during fat absorption. To resolve this difficulty Fourman suggests that chylomicrons are removed from the blood more slowly than are other forms of absorbed fat. The general trend of results supports Frazer's partition hypo-

thesis of fat absorption; and they are consistent with Stannus's suggestion that absorption of neutral fat in sprue is less affected than is absorption of split fat.

D. A. K. Black.

Aetiology of Steatorrhoea. FRAZER, A. C. (1947). *Brit. med. J.*, 2, 641.

The author emphasizes the importance of balance experiments rather than estimations of the percentage of fat in the faeces in any study of fat absorption. In a diet containing 50 g. of fat 95% or more is normally absorbed. Defective emulsification was found in biliary obstruction and hepatic cirrhosis and in cases of atrophic pancreatitis, but in no other cases of defective fat absorption. In sprue systemic lipaemia is absent, though intraluminar changes are normal and 60 to 70% fat is absorbed: in regional ileitis, on the other hand, lipaemia is present. Adrenalectomy prevents the characteristic loading of the cell with fat globules, and this may be corrected by adequate salt therapy. Adrenal cortical deficiency has been suggested as a factor in the sprue syndrome, but if symptoms suggestive of adrenal insufficiency, such as dehydration and low serum-sodium levels, are relieved by appropriate treatment the fat absorption defect remains unchanged.

Alternative routes are available for fat absorption, the triglycerides largely absorbed in the particulate form passing by the lymphatic route, the fatty acids by the portal vein. Variations in the proportion of fat passing by these two routes may occur without any great effect on the total fat absorbed. Thus in lymphatic obstruction the quantitative decrease in fat absorption is much less than might be expected.

Since active absorption of fat occurs from the mid-duodenum downward the long-chain triglycerides are absorbed mainly in the particulate form in the upper intestine and as fatty acid in the lower intestine. The tolerance of patients with fat-absorption defects to different dietary fats appears to vary considerably. The pH of the upper intestine may also affect the routes of absorption, since an alteration towards increased alkalinity would produce a decrease in particulate and an increase in fatty-acid absorption.

O. L. V. de Wesselow.

The Diagnosis of Pancreatic Disease by Enzyme Tests. MORRISON, L. M. (1947). *J. Lab. clin. Med.*, 32, 1107.

Specimens of duodenal contents were examined for proteinase, amylase, and lipase in fasting subjects and after the introduction of olive oil. In 6 cases of pancreatic carcinoma or cirrhosis these enzymes were consistently absent or were present only in traces. In 8 normal subjects the enzymes were occasionally but never repeatedly absent. In patients with non-organic digestive disturbances, or organic disease of the upper gastrointestinal tract, the findings were not diagnostic. In 10 patients with "chronic low-grade or moderate inflammation of the pancreas as revealed at operation" the enzyme content was not abnormal.

Marjorie Le Vay.

Antithyroid Activity of Ergothioneine in Man. ASTWOOD, E. B., and STANLEY, M. M. (1947). *Lancet*, 2, 905.

The previous findings that ergothioneine has an antithyroid effect similar to that of thiouracil, and that this substance occurs naturally in the blood have suggested that it might be involved in the regulation of thyroid

function. The substance, therefore, was administered to 5 normal persons in doses of 50, 200, and 400 mg. by mouth and 120 and 400 mg. intravenously, but without significant results.

Periodic Paralysis in a Patient with Exophthalmic Goiter Controlled by 6-Propylthiouracil. SEED, L. (1947). *W.J. Surg. Obstet. Gynec.*, 55, 640.

The author's patient was a young man who developed symptoms of exophthalmic goitre and periodic paralysis after the aircraft carrier in which he was surving had been sunk. His serum potassium level was not significantly lowered, and administration of 2 g. of potassium chloride four times a day had no effect on his condition. His symptoms were relieved by treatment with 6-propylthiouracil, and his condition was finally controlled by 50 mg. three times a day. *J. B. Hannah.*

Metabolic Studies in Periodic Paralysis. (Stoffwechseluntersuchungen bei paroxysmaler Lähmung.) JANTZ, H. (1947). *Nervenarzt*, 18, 360.

Nine patients (7 men, 2 women) suffering from periodic paralysis were studied in 8 years; altogether 68 attacks were observed. In every case the potassium content of the blood serum was lowered during an attack. The lowest value was 5.5 mg. per 100 ml. As soon as the paralysis receded, the potassium content of the blood serum reached the original level of about 20 mg. per 100 ml. Potassium chloride in 15- to 20-g. doses dissolved in water and given by mouth stopped every attack.

F. K. Kessel.

Potassium-calcium Metabolism in Chilblains. (Der Kalium-Calcium-Stoffwechsel bei der Perniosis.) SCHNEIDER, W., and ERASMY, H. (1947). *Arch. Derm. Syph.*, *Wien.*, 186, 137.

The normal ratio of potassium to calcium in the serum is taken as being 2 to 1. Twenty-three cases of chilblains were investigated and it was found that the average K.Ca ratio was 1:6; most of the values were below 2. The calcium was both relatively and absolutely increased; the potassium was absolutely increased but relatively diminished. The authors claim that in perniosis the preponderance of calcium in the blood varies with the arteriolar spasm, the latter being due to a low preponderance of sympathetic tone. It is not only a matter of the preponderance of one of the components; a failure of co-ordination is involved. It seems probable that perniosis is not due principally to the influence of cold but that is a disorder of the vegetative nervous system-hormone–electrolyte complex. *F. F. Jacobson.*

The Relative Importance of Dietary Sodium Chloride and Water Intake in Cardiac Edema. GORHAM, L. W., LESTER, D. E., WOLF, A. V., and SHULTZ, H. H. (1947). *Ann. intern. Med.*, 27, 575.

The authors investigated 22 patients with cardiac oedema who were placed on a low-salt diet containing less than 1 g. of sodium chloride daily, and also 8 patients who were given a diet containing 3 g. of sodium chloride. Daily fluid intake varied from 1,000 ml. to 7,500 ml. Sodium and chloride levels were determined in serum twice weekly and a 24-hour specimen of urine was also collected daily. The loss of sodium on the diet of less than 1 g. of sodium chloride was shown to be appreciably

greater than on the diet of 3 g. Very satisfactory clinical improvement was recorded in the majority of those patients on a diet containing less than 1 g. of sodium chloride. Some patients showed a urinary output exceeding the fluid intake. In general the clinical response of the patient appeared to depend on the ability to excrete sodium. In no case was there an adverse effect from the ingestion of large amounts of water when the diet was kept low in sodium chloride.

The object of the treatment is to maintain a low ratio of sodium intake to water intake, rather than a low sodium or a high water intake, and the restriction of salt is more effective in relieving cardiac oedema than is the forcing of fluid to a high level. *G. Hesketh.*

An Evaluation of Methods for Serum Proteins. BERRY, T. J., and PERKINS, E. (1947). *Amer. J. clin. Path.*, 17, 847.

The serum protein concentrate was estimated in 327 consecutive blood donors, comparing the turbidimetric, the micro-kjeldahl, and specific gravity methods. The range of serum-protein concentrations was from 5.2 to 7.7 g., with a mean of 6.33 g. and a standard deviation of 0.58 g. It is concluded that the turbidimetric method (Looney and Walsh, *J. biol. Chem.*, 1939, 130, 635) is the most suitable for use in clinical laboratories.

R. B. Lucas.

HAEMATOLOGY

A Simple Quantitative Formol-gel Reaction and its Relation to the Euglobulin and Gamma-globulin Content of Serum. (Een eenvoudige quantitatieve formolgelreactie en haar verband met het euglobulinegehalte en gamma-globulinegehalte van het serum.) VERHAGEN, B. A. (1947). *Ned. Tijdschr. Geneesk.*, 4, 3524.

Gel formation occurs after the addition of a calcium-formalin solution to serum if the γ-globulin content is raised above a certain critical level. A simple quantitative technique is described to estimate the amount of γ-globulin present. The results compare well with those given by electrophoresis.

A Study of 114 Patients with Haemorrhagic Syndromes Seen within One Year. (A Critical Study of Tests of Haemostasis.) (Etude de 114 cas de syndromes hémorragiques examinés en l'espace d'un an. (Etude critique des épreuves fonctionnelles de l'hémostase.) TZANCK, A., and SOULIER, J. P. (1947). *Rev. Hémat.*, 2, 429.

This long paper should be read by all interested in the haemorrhagic diatheses.

Thrombocytic Acroangiothrombosis. FITZGERALD, P. J., AUERBACH, O., and FRAME, E. (1947). *Blood*, 2, 519.
Thrombocytic Thrombocytopenic Purpura. SINGER, K., BORNSTEIN, F. P., and WILE, S. A. (1947). *Blood*, 2, 542.

These two papers refer to a rare fatal haemorrhagic state of acute or subacute course and unknown causation. The essential pathology is blockage of capillaries and arterioles by thrombi, mostly composed of platelets. Many organs are involved, and the clinical picture varies. The brain is often affected, and there may be mental symptoms. The spleen is sometimes palpable. A tendency to bleed is always present at some stage, and the platelet count is generally reduced.

Diagnosis of Thrombocytopenic Purpura. (Diagnostic des purpuras thrombopéniques.) DREYFUS, B., and SOULIER, J. P. (1947). *Rev. Hémat.*, 2, 305.

In 9 patients with primary thrombocytopenic purpura the total number of megakaryocytes in the bone marrow varied from 450 to 4,000 per million nucleated cells, compared with counts of 175 to 600 in 5 normal subjects and in 3 patients with constitutional athrombopenic purpura. There was less evidence of platelet formation by budding, and an increase in young forms of megakaryocytes in the cases with primary thrombocytopenia. In 6 patients with secondary thrombocytopenia the megakaryocytes were present in normal or reduced numbers.

Clotting Defect in Hemophilia : Deficiency in a Plasma Factor Required for Platelet Utilization. BRINKHOUS, K. M. (1947). *Proc. Soc. exp. Biol., N.Y.*, 66, 117.

Experiments are described in which platelet-free and platelet-rich plasmas were obtained from haemophiliacs and normals. Methylchlorosilane was employed to impart a non-wettable surface to all apparatus used so as to prevent the plasma from clotting during centrifugation. The author's results indicate that a plasma factor is required in addition to platelets for the proper coagulation of blood. This plasma factor is deficient in haemophilia.

Differences During Dicoumarol Therapy in the Quick and Russell Viper Venom Methods for Prothrombin Determination. WILSON, S. J. (1947). *Proc. Soc. exp. Biol., N.Y.*, 66, 126.

Prothrombin estimations using both Russell viper venom and rabbit brain as sources of thrombokinase were carried out in parallel on 7 patients receiving dicoumarol therapy. The author concludes that the changes observed when rabbit brain was used reflect the clinical condition more closely, and that the use of the viper venom may be dangerous, as the prothrombin level so measured may appear to be a safe one when, in reality, the patient is about to bleed.

Hemophilia. A Report on the Mechanism of the Development and Action of an Anticoagulant in Two Cases. CRADDOCK, C. G., and LAWRENCE, J. S. (1947). *Blood*, 2, 505.

Two haemophilic patients became refractory to treatment by whole blood transfusion or anti-haemophilic globulin. The patient's plasma contained an anticoagulant factor which was able to prolong the coagulation of normal plasma *in vitro*. The anticoagulant was of the nature of a γ-globulin and the authors suggest that this may have developed as an immune response to the many transfusions the patients had previously had.

Detection of Intravascular Clotting Tendency by Heparin Tolerance Principle. TUFT, H. S., and ROSENFIELD, R. E. (1947). *Amer. J. clin. Path.*, 17, 862.

The authors have modified the Waugh-Ruddick test. They estimate the delay in the clotting of venous blood *in vitro* produced by adding to it heparin at a concentration of 0.01 mg. per ml. blood. In a group of 25 patients with various conditions of thrombosis or embolism the clotting times were 30 minutes or less, compared with 60 minutes using normal blood. It was shortened also in 15 out of 25 patients with myocardial infarcts.

A Modification of the Waugh-Ruddick Test for Increased Coagulability of the Blood and its Application to the Study of Postoperative Cases. SILVERMAN, S. B. (1948). *Blood*, 3, 147.

Using a modified Waugh-Ruddick test (coagulation in the presence of a small standard concentration of heparin) in which recalcified plasma was used in place of whole blood, a postoperative increase in coagulability of the blood was demonstrated which began within 24 hours and lasted from 7 to 14 days after operation.

Prothrombin, Fibrinogen, and Protein Content, and Viscosity of the Blood in Thromboangiitis Obliterans. (Comportamento della protrombina, fibrinogeno, viscosimetria, e proteinemia nella tromboangioite obliterante di Burger.) SPOSITO, M., GIANNICO, O., and MARRAZZA, P. (1947). *Policlinico, sez. prat.*, 54, 1235.

The authors studied 29 cases of Buerger's disease and claim that the prothrombin level was raised by an average of 15%. In 22 patients the fibrinogen averaged 570 mg. per 100 ml. The average total plasma protein was 8.1 g. per 100 ml. The authors believe that similar small rises in prothrombin and fibrinogen are found in many conditions where blood vessels are diseased. The use of dicoumarol is suggested.

Streakiness of Blood Films. Relation to Sedimentation-Rate and Plasma-Fibrinogen. BOVERI, R. M., WATERFIELD, R. L., and NEWMAN, T. H. (1947). *Lancet*, 2, 831.

Streakiness in blood films spread on slides is due to an excess of plasma fibrinogen. This phenomenon is associated with a raised sedimentation rate, but not all bloods which undergo rapid rouleaux formation and have a raised sedimentation rate make streaky films. This is because a raised plasma globulin which increases rouleaux formation and rate of sedimentation does not cause streakiness.

The Action of Cobalt in Man. (Kobaltwirkungen am Menschen.) WEISSBECKER, L., and MAURER, R. (1947). *Klin. Wschr.*, 24/25, 855.

This paper is a preliminary report on the action of cobalt on blood formation in man. Increases in reticulocytes and erythrocytes were noted in normal subjects and in anaemia due to haemorrhage and infection. The authors suggest that cobalt acts as a stimulus to erythropoiesis and indirectly affects the formation of haemoglobin. Cobalt is a toxic substance, and minor effects are described after the oral or intravenous administration of small doses.

Genuine Aplastic Anaemia with Complete Absence of Erythroblasts. (Über eine isolierte aplastische Anämie mit vollständigem Fehlen der Erythroblasten (Erythroblastophthise).) BEGEMANN, H. (1947). *Klin. Wschr.*, 24/25, 850.

The author describes the incidence in a man aged 59 of a rare disorder—primary aplastic anaemia affecting erythropoiesis only. The formation of leucocytes and platelets was unaffected.

The Development and Progression of Subacute Combined Degeneration of the Spinal Cord in Patients with Pernicious Anemia Treated with Synthetic Pteroylglutamic (Folic) Acid. Ross, J. F., Belding, H., and Paegel, B. L. (1948). *Blood*, 3, 68.

The authors describe the development and progression of subacute combined degeneration of the cord in 11 of 25 patients maintained in satisfactory haematological remission with oral folic acid. The possibility that folic acid has actually a deleterious effect on the nervous system is discussed.

Changes in the Bone Marrow in Megaloblastic Anemias of Infancy Before and After Folic Acid Therapy. Zuelzer, W. W., Newhall, A., and Hutaff, L. (1947). *J. Lab. clin. Med.*, 32, 1217.

The authors studied the bone marrows of 15 children suffering from the megaloblastic anaemia of infancy. In some a primitive megaloblastic picture was seen, in others a less typical picture with cells intermediate in appearance between megaloblasts and normoblasts. In those cases with a typical megaloblastic marrow intermediate types were conspicuous for a day or two after folic-acid therapy and were then themselves replaced by normoblasts. This paper should be read by all interested in megaloblasts.

Refractory Megaloblastic Anemia. Davidson, L. S. P. (1948). *Blood*, 3, 107.

The author describes cases of anaemia with megaloblastic marrows partially or completely refractory to parenteral liver therapy. Some were examples of anaemia associated with sprue, pregnancy, or malnutrition. In 25 patients no cause was obvious. Ten of them responded slowly to numerous injections of liver extract and blood transfusions, the rest to proteolysed liver or folic acid. The superior value of oral as contrasted with parenteral liver in the treatment of refractory patients is discussed.

Experimental Study on the Localization of Castle's Intrinsic Factor in the Human Stomach. Antianemic Effect of Powdered Human Fundus and Pylorus. Landboe-Christensen, E., and Plum, C. M. (1948). *Amer. J. med. Sci.*, 215, 17.

Extracts of the fundus of human stomachs were more effective in treating pernicious anaemia than were extracts of the pyloric region. In the case of the hog the reverse is true.

Acute Hemolytic Anemia in Primary Atypical Pneumonia Produced by Exposure and Chilling. Colmers, R. A., and Snavely, J. G. (1947). *New Engl. J. Med.*, 237, 505.

Anaemia rapidly developed in a woman with clinical and radiological signs of primary atypical pneumonia. A cold-agglutinin was present, titre 1:1280 at 7° C., but still active (titre 1:5) at 37° C. This was thought to be responsible for the anaemia.

A New Antibody in Serum of Patients with Acquired Hemolytic Anemia. Sturgeon, P. (1947). *Science*, 106, 293.

The authors claim to have demonstrated by means of the "indirect Coombs' Test" an antibody free in the sera of three patients with acquired haemolytic anaemia,

a distinctly unusual finding. In one case the titre of antibody was 1:4,000. The antibody could be eluted off the patient's red cells by incubating them at temperatures between 37° C. and 56° C.

The Cryptogenic Acquired Haemolytic Anaemias. Fisher, J. A. (1947). *Quart. J. Med.*, 16, 245.

This is mainly a clinical review of 18 cases, in 4 of whom liver dysfunction was present. The results of splenectomy and of transfusions are considered.

Determination of Haemoglobin. II. The Haldane Haemoglobin Standard Compared with Iron and Gasometric Estimations. King, E. J., Gilchrist, M., Wooton, I. D. P., Donaldson, R., Sisson, R. B., MacFarlane, R. G., Jope, H. M., O'Brien, J. R. P., Peterson, J. M., and Strangeways, D. H. (1947). *Lancet*, 2, 789.

As a result of thorough large-scale investigations it has been found that the Haldane colour standard of British Standards Institution specification corresponds to 14.8 g. Hb. per 100 ml. of blood. If this figure is accepted, the supposed differences in the normal haemoglobin range between Britain and America as suggested by pre-war surveys can be explained. In reality, no differences probably exist.

Effect of β-Chlorethylamine Hydrochlorides in Leukaemia, Hodgkin's Disease, and Polycythaemia Vera. Report on Eighteen Cases. Wilkinson, J. F., and Fletcher, F. (1947). *Lancet*, 2, 540.

Eleven patients with chronic leukaemia, four with Hodgkin's disease, and three with polycythaemia vera were studied. There was a satisfactory reduction in the leucocyte level, splenomegaly, and lymph-adenopathy in the leukaemias, but in only 5 patients any rise in haemoglobin level. A striking improvement resulted in 3 of the 4 patients with Hodgkin's disease, but only 1 of the cases of polycythaemia showed any improvement. Except possibly in Hodgkin's disease, these dangerous and toxic compounds do not seem to have any advantage over x-ray therapy.

Familial Idiopathic Methaemoglobinaemia. Five Cases in One Family. Gibson, Q. H., and Harrison, D. C. (1947). *Lancet*, 2, 941.

Quantitative estimations of methaemoglobin gave figures of 1.9 to 5.1 g. per 100 ml. blood in the five affected subjects. Treatment with methylene blue reduced these figures to 0.1 to 1.3 g. per 100 ml. It is thought that deficiency of coenzyme factor I is responsible for the presence of methaemoglobin, and that all the red cells contain a proportion of this pigment. Methylene blue is considered to catalyse the enzymic reduction of methaemoglobin.

The Relating O$_2$ in Bone Marrow Blood to Post-haemorrhagic Erythropoiesis. Grant, W. C., and Root, W. S. (1947). *Amer. J. Physiol.*, 150, 618.

Reduced oxygen tension has been held to be a stimulus for increased erythropoiesis. A single large haemorrhage resulted in a transient reduction in oxygen tension in the blood from the humerus of dogs, but repeated small haemorrhages were without effect. Both procedures were equally effective as stimuli of erythropoiesis. The authors' experiments thus suggest that anoxia has no direct stimulatory action on the bone marrow.

Raynaud's Syndrome Originating from Reversible Precipitation of Protein. (In English.) HANSEN, P. F., and FABER, M. (1947). *Acta med. scand.*, 129, 81.

The authors describe the onset of Raynaud's syndrome due apparently to the presence of an abnormal plasma euglobulin which was reversibly precipitated by cold. Multiple arterial embolisms were thus produced. The circulation could be restarted by applying warmth. The pathological diagnosis was aleukaemic plasma-cell leukaemia.

Some Investigations of Erythropoiesis in Human Bone-marrow Cultivated in Various Media. (In English.) PLUM, C. M. (1947). *Acta physiol. scand.*, 14, 383.

This paper deals with the cultivation of aspirated normal and pathological bone marrow in Locke's solution with additions of serum, liver extract, folic acid, etc. It should be read by all interested.

Cold Hemagglutinins in Sickle-Cell Anemia. McSWEENEY, J. E. J., MERMANN, A. C., and WAGLEY, P. F. (1947). *Amer. J. med. Sci.*, 214, 542.

Sixty per cent of sera from 30 cases of sickle-cell anaemia contained cold agglutinins at a titre > 1:40; in 36% the titre was > 1:80. Three out of 30 control sera contained cold agglutinins of titre 1:40.

The Prozone Phenomenon in Rh Blocking Serums. HATTERSLEY, P. G., and FAWCETT, M. L. (1947). *Amer. J. clin. Path.*, 17, 695.

A high degree of immunization is reported in 3 cases where a marked prozone prevented the recognition of hyperimmune or incomplete antibody by a single-tube technique, even when the test cells were suspended in 30% bovine albumin. Investigation of the causes of zone phenomena elicited the following facts. The zones were relatively unaffected by: (1) variation in cell concentration; (2) speed of centrifuging; (3) presence or absence of complement. They were influenced by: (1) temperature of incubation (prozone was most marked at 37° C., weak or absent at room temperature); (2) time of incubation prozone increased with prolonged incubation. The authors concluded that sera should never be heated, and that the bovine albumin test of Diamond and Denton (*J. Lab. clin. Med.*, 1945, 30, 821) can be improved by centrifuging both immediately before and after incubation, the cell deposits being examined microscopically after each spinning. (Sera from cases with such suggestive histories should be examined by the indirect sensitization test of Coombs and others.) *John Murray.*

Studies on the Conglutination Reaction, with Special Reference to the Nature of Conglutinin. WIENER, A. S., HURST, J. G., and SONN-GORDON, E. B. (1947). *J. exp. Med.*, 86, 267.

The authors have tested various blood-protein-containing preparations for their power of agglutinating Rh-positive cells which have absorbed incomplete Rh antibody. Albumin, fibrinogen, and β-globulin make up conglutinin, or X-protein. One part of albumin added to 5 parts of human plasma notably increases the conglutination activity of the latter.

The conglutinin titre of foetal blood is low, but quickly increases after birth. Wiener recommends removing part of the donor's plasma before exchange transfusions so as to reduce its conglutinin content.

Haemolytic Icterus (with Special Reference to its Pathogenesis). (Icterus haemolyticus. Med saerlig henblikk på patogenesen.) OWREN, P. A. (1947). *Tidsskr. norske Laegeforen*, 67, 665.

The author reports interesting serial blood and bone marrow findings which suggest that in familial haemolytic icterus the "haemolytic crises" are due to temporary marrow aplasia rather than to increased haemolysis (see also *Blood*, 1948, 3, 231). The mechanisms of erythrocyte destruction in the familial and acquired types of haemolytic anaemia are also considered and contrasted.

Erythropoiesis in Chronic Erythroblastosis in Adults. (L'erythropoïèse dans les erythroblastoses chroniques de l'adulte.) OLMER, J., and GASTAUT, H. (1947). *Ann. Méd.*, 48, 458.

This interesting, obscure, and not very uncommon disorder is generally referred to in British and American literature as myelosclerosis or chronic non-leukaemic myclosis. Anaemia, erythroblastaemia with some primitive granulocytes, a greatly enlarged spleen, moderate enlargement of the liver, and an atrophic or fibrotic marrow are the main characteristics. Its course is a prolonged one.

Studies in Erythroblastosis Fetalis. I. Activation of the Incomplete Rh Antibody by the Blood Serum of Full-term and Premature Newborn Infants. WITEBSKY, E., RUBIN, M. I., and BLUM, L. (1947). *J. Lab. clin. Med.*, 32, 1330.

Studies in Erythroblastosis Fetalis. II. Investigations on the Detection of Sensitization of the Red Blood Cells of Newborn Infants with Erythroblastosis Fetalis. WITEBSKY, E., RUBIN, M. I., ENGASSER, L. M., and BLUM, L. (1947). *J. Lab. clin. Med.*, 32, 1339.

The authors have found that cord sera from newborn infants and in particular from premature infants are generally much less active than the sera of adults in causing agglutination of Rh-positive cells which have been subjected to "incomplete" or blocking Rh antibody. The sera of infants 24 to 48 hours old were more active than cord sera. It is suggested that this increase in serum activity developing after birth may be connected with the delayed post-natal onset of haemolysis sometimes observed in haemolytic disease of the newborn.

In the second paper the authors recommend suspending the cells suspected of sensitization in adult serum, preferably on a tile.

Breast-feeding in Erythroblastosis Foetalis. CATHIE, I. A. B. (1947). *Brit. med. J.*, 2, 650.

The author has demonstrated that Rh antibody in serum is not destroyed by 1 hour's incubation in infant's gastric juice. However, there was no evidence of any absorption when breast milk containing antibodies or high-titre Rh anti-serum was fed to Rh-positive infants or to an adult. It is concluded that it is safe and justifiable to breast-feed infants with haemolytic disease of the newborn even if the mothers' milk contains Rh antibodies.

Multiple Myeloma. A Survey Based on Thirty-five Cases, Eighteen of which came to Autopsy. LICHTENSTEIN, L., and JAFFE, H. L. (1947). *Arch. Path., Chicago*, 44, 207.

In this review clinical, biochemical, haematological, and pathological features are considered. In half the cases serum calcium is raised. Calcium granules are

deposited in the kidneys and renal failure may follow. Secondary hyperplasia of the parathyroids may develop, but was not seen in this series. Hyperproteinaemia, and more specifically hyperglobulinaemia, is seen in about half of the cases. Bence-Jones proteinuria was present in 10 of 26 cases; it is very variable in quantity and occurrence. The serum uric acid, as in leukaemia, is increased. Amyloid is mentioned as a complication in 10% of cases, and the disease is considered to be related to the leukaemias. The plasma cells, which vary in maturity, are thought to be abnormal neoplastic haematic cells.

MORBID ANATOMY AND HISTOLOGY

Cranial Trauma and Extrapyramidal Involvement : Cerebral Changes Simulating Those of Anoxia. A Clinico-pathologic Report of Three Cases. MALAMUD, N., and HAYMAKER, W. (1947). *J. Neuropath. exp. Neurol.*, 6, 217.

Three cases of head injury are described in which the chief lesions were in the lenticular nuclei. In 1 case there was also softening of the substantia nigra and pseudo-laminar cortical necrosis, and most of the Purkinje cells of the cerebellum had disappeared. Cessation of respiration during anaesthesia for reduction of fractures may have been a causative factor in this case. Fat embolism was not found. The authors suggest that similar anoxic lesions may be the basis of cases of post-traumatic Parkinsonism. *J. G. Greenfield.*

The Use of the Smear Technique in the Rapid Histological Diagnosis of Tumors of the Central Nervous System. Description of a New Staining Method. MORRIS, A. A. (1947). *J. Neurosurg.*, 4, 497.

The author reports favourably on the use of a special eosin-methylene-blue staining technique for the rapid diagnosis of cerebral tumours by the smear biopsy method. (For details of the technique, which is not his own, the author's original article should be consulted.) By this technique smears can be examined under the microscope within 30 to 40 seconds of being made. The author has used the technique in a series of 116 tumours of the central nervous system. He considers the method of great value, although it can, at best, give only a presumptive diagnosis, which should be confirmed whenever possible by routine histological techniques. *J. G. Greenfield.*

Positive and Negative Aspects of Hypothalamic Disorders. (In English.) BROUWER, B. (1947). *Proc. Akad. Wet. Amst.*, 50, 1038.

Ten cases with symptoms attributable to lesions of the hypothalamus were compared with experimental hypothalamic lesions. Discrepancies were found in all types of lesion. Three cases of diabetes insipidus showed bilateral destruction of the supra-optic nuclei as was expected from the experimental work, but one case with similar destruction did not have diabetes insipidus. All the other expected lesions are discussed.

Primitive Malignant Tumours of the Nose and Pharynx. (Primitive maligne Geschwülste der Nasenund Rachengegend.) LÜDIN, M. (1947). *Prat. oto-rhino-loryng., Basel*, 9, 148.

The author discusses the literature on those tumours most often found in the nasopharynx or oropharynx which invade the lymph nodes early and produce distant metastases. Because of the frequent findings of an epithelial syncytium, in the meshes of which are embedded masses of rounded cells, these tumours have been described as carcino-sarcomata, lympho-epitheliomata, and reticulo-cell sarcomata. The prevailing tendency is to consider these embryonal tumours as sarcomata. Some resemble undifferentiated carcinomata, others lympho-sarcomata, and a third group are so primitive that no distinction between epethelial or mesenchymatous tissue is possible.

The clinical notes and detailed histology of 21 cases are given. The author considers that those which can with certainty be said to arise from epithelium may be called carcinomata, and those which can be said to arise from a mesenchymal matrix may be called sarcomata, but that the primitive undifferentiated tumours should be called "meristomata." *Stephen Suggit.*

The Histology of the Irradiated Larynx. PRICOLO, V., and PIZZETTI, F. (1947). *Radiolog. med., Torino*, 33, 586.

The authors find that many of the vascular and cartilaginous changes previously attributed to irradiation of this region are to be found in normal specimens between the ages of 30 and 50. Comparison of controls with non-irradiated and irradiated carcinomatous larynges shows that the only effect attributable to x-rays is round-celled infiltration of the mucosa and sub-mucosa.

Primary Carcinoma of the Trachea. ELLMAN, P., and WHITTAKER, H. (1947). *Thorax*, 2, 153.

The authors summarize and discuss the literature on tracheal tumours and describe a case shown at necropsy to be one of carcinoma of the trachea. The patient complained of hoarseness, largely due to recurrent laryngeal palsy, and had signs deriving from transient collapse of the lower lobe of the left lung. The tumour arose on the posterior wall of the trachea in its lower third. Part of the growth, nearly 2 cm. in diameter, projected into the lumen; a much larger mass, measuring some 5 × 3 cm., extended into the mediastinum. Para-tracheal glands and those at the bifurcation of the trachea were involved by metastases, but no other spread of the tumour was found. Section of the growth showed it to be a squamous-celled carcinoma. *W. D. W. Brooks.*

An Unusual Hamartoma (So-called Chondroma of the Lung). SIMON, M. A., and BALLON, H. C. (1947). *J. thoroc. Surg.*, 16, 379.

So-called chondromata of the lung are not pure cartilaginous tumours but contain mixtures of elements normally encountered in the bronchial wall and are therefore more properly referred to as hamartomata or hamartoma chondromatosum pulmonis. Such a tumour is usually considered benign and slow-growing. It may, however, undergo malignant change. The tumour here described is unusual because of its enormous size and the fact that it caused symptoms and radiologically simulated bronchiogenic carcinoma. *George A. Mason.*

The Myoepithelium in Certain Tumours of the Breast. BIGGS. R. (1947). *J. Path. Bact.*, 59, 437.

The author describes examples of various neoplastic and hyperplastic lesions of the human and canine breast in which she believes myoepithelial cells to be demonstrable.

Is the Brenner Tumour always a Benign Neoplasm? (Ist der Brenner-tumor immer eine gutartige Neubildung?) DUBRAUSZKY, V., and MASSENBACH, W. VON (1947). *Zbl. Gynäk.*, 69, 370.

The authors believe that the Brenner tumour originates from the coelomic epithelium, and that some of the solid carcinomata and cystic adenocarcinomata of the ovary may originate in a Brenner tumour. They describe the tumour from a woman aged 70, in which the medullary part of the tumour, as distinguished from the cystic portions, showed definite signs of malignancy, and the transition from benign to malignant epithelium could be followed. The Brenner tumour does not produce any oestrogenic hormone, and bleeding in this case was caused by a polypoid hyperplasia of the endometrium. This would appear to be the first described case of a definitely malignant Brenner tumour.

Composition and Structure of the Liver Cell in Pregnancy. KOSTERLITZ, H. W., and CAMPBELL, R. M. (1947). *Nature, Lond.*, 150, 676.

The desoxyribonucleic-acid content of the liver of pregnant rats was shown to be significantly increased by the third week of gestation and was greater than could be accounted for by the increase in maternal body weight. The total amount was related to the sum of maternal and foetal body weights and was independent of the diet. A specific rise of 40 to 45% in the ratio of ribonucleic to desoxyribonucleic acids also occurred; this was not due to dietary changes during pregnancy and was correlated with foetal body weight. The increased ribonucleic-acid content was particularly apparent in the periportal area. Glycogen content of the liver was much decreased.

J. Dawson.

Glycogen Formation and Deposition in the Human Liver. (Über Glykogenbildung und Glykogenablagerung in der menschlichen Leber.) EGER, W., and KLÄRNER, G. (1948). *Virchows Arch.*, 315, 135.

Estimation of glycogen in the liver in 250 necropsies, 19 of them performed on subjects who had died suddenly ("normal cases"), and the rest on subjects who had died after illness of longer duration, was carried out by Pflüger's method. The values were calculated in terms of wet weight, dry weight, or "dry protein weight"—that is, dry weight minus fat and glycogen. The values for glycogen were higher in "normal cases," averaging 7.09% against 2.04% of dry weight in the other cases. No antagonism was found between the quantities of fat and glycogen. The results of histological estimation by the methods of Bauer and Best were not in accord with the chemical determinations. In "normal cases" the glycogen is located in the central parts of the lobules. Deposition in the periphery of the lobules and in the nuclei is explained as a sign of damaged function of the liver. It is suggested that glycogen is normally built up in the nucleus, then goes into the body of the cell for storage; this hypothesis would be in accordance with the fact that livers rich in glycogen contain more uric acid (3,232 mg. per 100 g. of dry protein weight against 1,437 mg.). *O. Neubauer.*

Gaucher's Disease Without Splenomegaly. MORGANS, M. E. (1947). *Lancet*, 2, 576.

A family affected by Gaucher's disease is described. In the father the diagnosis was first made when he was 67 years old; sternal puncture revealed Gaucher cells and radiographs showed translucent areas in bone. There was enlargement of the liver, but the spleen was not palpable. Two of his 3 children, a man aged 23 and a girl aged 14, had radiological evidence of the disease, and in the third child, a boy aged 12, the radiological appearances were not conclusive. The family history indicated that the disease may have occurred in the two previous generations. The series is remarkable in three ways: the absence of splenomegaly, the appearance of the disease in successive generations, and the survival of 1 patient in active life to a good age.

K. Black.

The Hepatitis of Hyperthermia. Report of a Fatal Case. BRAGDON, J. H. (1947). *New Engl. J. Med.*, 237, 765.

A fatal case of hepatitis and hepato-renal syndrome is described in a patient suffering from sulphonamide-resistant gonorrhoea who was treated by artificial fever. The pathogenesis is discussed.

Gelatin Nephrosis. Renal Tissue Changes in Man Resulting from the Intravenous Administration of Gelatin. SKINSNES, O. K. (1947). *Surg. Gynec. Obstet.*, 85, 563.

The author has studied the effects of gelatin given intravenously in 8% saline as a treatment for peripheral vascular collapse; 23 patients so treated came to necropsy, most of them having been treated surgically for malignant disease within the abdomen. The kidneys affected by gelatin were swollen and the cut surfaces bulged. Microscopically the kidneys were found to be the seat of a change similar to the nephrosis caused by sucrose. The most important single factor related to these changes was the time interval between gelatin administration and death. All patients examined within 67 hours of their last gelatin injection showed gelatin nephrosis; in patients who died more than 120 hours after the last gelatin injection these changes were not seen. *I. Aird.*

Zinc Sulphate Flotation of Faeces. ELSDON-DEW, R. (1947). *Trans. R. Soc. trop. Med. Hyg.*, 41, 213.

The zinc sulphate flotation technique for the concentration of ova and cysts in faeces (Faust *et al.*, 1938) is described.

In 1,539 specimens, mostly from cases of suspected amoebiasis, this technique was compared with a direct faecal film. A considerable increase in positive results was obtained with most intestinal parasites and protozoal cysts. Thus, with *Entamoeba histolytica, Bact. coli, E. nana*, and *Iodamoeba bütschlii*, the ratio

$$\frac{\text{percentage positive results with flotation technique}}{\text{percentage positive results with direct film}}$$

was 175.5, 170.6, 315.2, and 577.8 respectively; with *Giardia lamblia* and *Chilomastix mesnili*, 500, and 300; with *Ascaris lumbricoides, Trichuris trichura*, and *Ancylostoma*, 122.8, 201.9, and 538.2. The ova of *Taenia* and *Schistosoma mansoni* did not float well, but could often be found in the sediment after the final spinning. This method should only be used, however, as an adjunct to the direct film examination, which reveals the trophozoites in addition. *J. L. Markson.*

Thrombosis of the Hepatic Veins. The Budd-Chiari Syndrome. THOMPSON, R. B. (1947). *Arch. intern. Med.*, 80, 602.

The author reviews 95 cases of thrombosis of the hepatic veins collected from the literature and reports a

further 2 cases. He suggests that the term "Budd-Chiari syndrome" should be reserved for cases in which there is evidence of gross blockage of the hepatic veins; cases without such evidence should be classified as "thrombosis of hepatic veins."

In both groups there were three main sites of obstruction: the hepatic veins, their ostia, and the inferior vena cava. Severe venous engorgement in the acute stage resulted in central lobular necrosis. Later cirrhotic changes and nodular hyperplasia were common. Portal thrombosis was rare and usually a terminal event. Venous engorgement, severe in the spleen and of varying degree in the intestines, was usually found. The age incidence varied from 17 months to 61 years, the average being 34. Males were affected more often than females. There were 8 cases of polycythaemia rubra vera and 4 pregnant women. A probable cause was phlebitis of the hepatic veins, which may be part of a more generalized vascular disease. In the full picture epigastric pain of variable severity was usually the initial symptom. Vomiting occurred in about a quarter of the cases and was severest at the onset. Ascites and marked hepatic enlargement were usual. The liver was generally tender. Splenic enlargement was much less frequent. The development of venous collaterals was one of the most important signs. Oedema of the legs developed in about half the cases. Jaundice was slight or latent. The author suggests that the diagnosis of minor thrombosis should be kept in mind in cases of obscure pain in the upper abdomen, especially where there is evidence of liver damage. As a rule the disease was of short duration but in a small group it lasted for 10 to 28 years. Of 9 patients who underwent operation, 8 died soon afterwards. *J. B. Mitchell.*

Pathologic Significance of the Ductus Arteriosus. Its Relation to the Process of Arteriosclerosis. BLUMENTHAL, L. S. (1947). *Arch. Path.*, 44, 372.

The histological changes associated with closure of the ductus arteriosus range from replacement of the normal smooth muscle by fibrous tissue to formation of localized areas of necrosis. It is suggested that these changes are the result of anoxic conditions produced by the contraction of smooth muscle of the wall of the ductus. These changes are discussed in relation to the wider problem of arteriosclerosis and to the possibility that localized anoxaemia is the fundamental mechanism in both processes. (There is a useful bibliography.)
R. H. D. Short.

Cholesterol Crystal Embolism of Minor Organ Arteries and its Consequences. (Cholesterinkrystallembolie kleiner Organarterien und ihre Folgen.) MEYER, W. W. (1947). *Virchows Arch.*, 314, 616.

A new form of embolism—embolic transport of cholesterol crystals from atheromatous ulcers of the aorta into small arteries of different organs—is described. The crystals are easily detected in the lumen of arteries of kidneys, brain, and meninges in frozen sections. Two cases are reported in detail. The embolized cholesterol crystals lead to a particular form of arteritis with numerous giant cells, and later to obliteration of the arteries, with subsequent changes in the tissues, such as scars on the surface of the kidneys and circumscribed softening of the brain. *O. Neubauer.*

Pulmonary Embolism by Amniotic Fluid. Report of 3 Cases with a New Diagnostic Procedure. GROSS, P., and BENZ, E. J. (1947). *Surg. Gynec. Obstet.*, 85, 315.

Three new cases are presented of a rarely described form of embolism, due to amniotic fluid. Previously 12 cases have been described. In all cases the diagnosis has been made at necropsy, generally after histological examination.

Summary of the necropsy findings shows death to have been asphyxial without gross lesions. The diagnosis is made by finding emboli rich in polymorphonuclear leucocytes, mucin, bile-stained debris (meconium), epithelial squamae, lanugo hair, and granular debris with or without fatty elements in histological preparations of the arteries, arterioles, and capillaries of the lungs. Blood from the right heart or the inferior vena cava (which may be obtained when permission for necropsy is withheld) may show three strata (instead of two) after centrifugation. The presence of the third (top) layer is considered pathognomonic. The top layer is separated. After fixation in alcohol, Zenker's fluid, and formol, sections stained by mucicarmine and Mallory's phosphotungstic acid and haematoxylin are prepared. Constituents of meconium and amniotic fluid are demonstrable.

In these 3 cases (all in multiparae) severe post-partum (1 intra-partum) irreversible shock was followed by death within 65 minutes. No case could be classified as one of difficult labour. Routine aspiration of blood (see above) should be undertaken in all obstetric deaths. (Infant weights are not recorded.) *Magnus Haines.*

Primary Hypertrophy and Hyperplasia of the Parathyroid Glands as a Cause of Hyperparathyroidism. ROGERS, H. M., and KEATING, F. R. (1947). *Amer. J. Med.*, 3, 384.

The authors review 22 cases from the literature and add 4 from the Mayo Clinic. On analysis there appears to be some curious connexion between hyperparathyroidism and duodenal ulcer; this lesion was present in 3 of the 4 cases here reported, and the authors have noted the co-existence of duodenal ulcer in approximately one-third of all the patients with hyperparathyroidism seen at the Mayo Clinic. They state that "this association seems too frequent to be coincidence but one can only conjecture as to its meaning."

The Intestinal Phase of Human Trichinosis. STRYKER, W. A. (1947). *Amer. J. Path.*, 23, 819.

Living adult trichinae, including gravid females, were demonstrated in the intestine of a fatal case of human trichiniasis 54 days after ingestion of infected pork—the longest period of persistence of adult trichinae in the human intestine thus far reported. The possibility of continued release of larvae over an even longer period must be taken into account in the therapeutic management of trichiniasis.

Endemic (Murine) Typhus. Report of Autopsy Findings in Three Cases. BINFORD, C. H., and ECKER, H. D. (1947). *Amer. J. clin. Path.*, 17, 797.

The authors give a detailed description of 3 fatal cases of endemic typhus. An acute interstitial myocarditis, with mononuclear and plasma-cell infiltration but with no apparent damage to the cardiac muscle fibres, was present in all, and was probably a major factor contributing to death. In 2 cases there was severe interstitial orchitis, and in 1 case skin lesions, closely resembling those found in epidemic typhus, showed a cellular perivascular infiltration with oedema and obliteration of several vessels by inflammatory cells or by thrombi. No typical typhus lesions were found in brain or lungs.

Comparative Morphological Investigations on the Mammary Glands of Mice of Different Origin. Investigations Concerning the Morphological Test of the So-Called Milk Factor. (In Russian.) POGOSYANTS, E. E. (1947). *Arkh. Patol.*, 9, No. 2, 64.

Experiments were carried out to devise a morphological test for the so-called milk factor. Mammary glands were examined by the whole mount technique in in-bred strains of varying incidence of breast cancer and the results confirmed the findings of other workers on the differences in structure in mammary glands between high and low breast cancer strains manifested by greater alveolar proliferation with nodules on hyperplastic tissue. In addition, 35 low-incidence strains received the milk factor whilst 5 to 25 days old, and 10 of these developed breast tumours; 14 receiving the extract when 30 to 45 days old did not develop tumours. The author concludes that the morphological examination of mammary glands of mice by the whole mount technique is a satisfactory test for the presence of the milk factor in breeding females not less than 6 months old.

The Antigenic Relationship of Lymphogranuloma Venereum and Psittacosis by Skin Test in Humans. POLLARD, M., and WITKA, T. M. (1947). *Tex. Rep. Biol. Med.*, 5, 288.

A psittacosis antigen suitable for carrying out skin tests was prepared, and tests were carried out with this antigen and with lygranum on patients with venereal disease in an army hospital. All 8 confirmed cases of lymphogranuloma venereum gave a severe reaction to the psittacosis antigen; 6 suspected cases, of which 2 were confirmed serologically, gave indefinite reactions; while 5 cases of penile ulcer, 8 cases of syphilis, and 3 suspected cases of syphilis were negative to both skin antigens. Lymphogranuloma venereum and psittacosis thus show an antigenic relation in the skin test, and this test is evidently not suitable for diagnosis when there is any possibility of the diseases co-existing. *D. J. Bauer.*

THE ERRORS OF SOME HAEMATOLOGICAL METHODS AS THEY ARE USED IN A ROUTINE LABORATORY

BY

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The quantitative measurements made in a haematological laboratory assess the magnitude of deviations from the normal, and successive readings indicate either the response to treatment or the natural progress of the disease. These figures can be of little value unless the range of chance variation that several laboratory workers may record in one blood sample is less than the difference that the clinician requires to distinguish. It has been usual for the standard of precis on of a method to be set by one expert, whose results, while measuring the minimum error, may bear little relation to ordinary routine practice. In the present study an attempt was made to assess the errors of some common haematological methods as they are used as a routine in laboratories. Those taking part in the experiments were laboratory technicians and doctors of average skill, who carried out the investigations carefully; any gross technical inaccuracies can be excluded. It was hoped that the experiments might suggest which techniques were likely to give the most generally reliable results. The methods studied were: haemoglobin estimation, red cell count, and haematocrit, from which the errors of the colour index, mean corpuscular volume, and mean corpuscular haemoglobin concentration could be calculated. Other methods included were the mean cell diameter derived from the Price-Jones curve, reticulocyte count, platelet count, whole blood coagulation, and red cell fragility. The white cell count was not considered because, since the work of Berkson and others (1935, 1940) it is widely recognized that this investigation has a large error.

Technique*

Red cell counts.—These were made by diluting the blood in Hayem's fluid to 1/200 and counting eighty small squares in a Neubauer counting-chamber.

Haemoglobin.—This was estimated by two methods:

1. The Haldane method, using a standard calibrated by the National Physical Laboratory. The tubes were matched against a uniformly illuminated screen. 14.7 g. haemoglobin were taken to represent 100 per cent (King and others, 1947).

2. A neutral grey photometer method using an instrument designed by Duffie (1945). In this method 0.02 ml. of blood was pipetted into approximately 2 ml. of disulled water in a cylindrical tube of standard diameter. The oxyhaemoglobin content was measured in terms of the optical density of the solution in comparison with a neutral grey wedge calibrated in grammes of haemoglobin, 14.7 g. being taken to represent 100 per cent.

Packed cell volume.—This was measured in Wintrobe's tubes, using oxalated venous blood in which the proportion of oxalate recommended by Wintrobe (1946) was used.

Price-Jones curves.—These were constructed using a projecting microscope and a high-power objective in a dry system giving a magnification on the screen of 2,000. The cells were measured directly, and each mean cell diameter was recorded with no preliminary outlining in pencil.

Platelets.—Platelets were counted by two methods:

1. Dameshek's method (1932). In this method the platelets in capillary blood, stained with brilliant

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* The determinations of red cell count, haemoglobin, packed cell volume, and mean cell diameter were made from the same oxalated venous blood sample. The reticulocyte counts and platelet counts were made from capillary blood.

TABLE I

THE ERRORS OF SOME HAEMATOLOGICAL METHODS AS THEY ARE USED IN THE ROUTINE LABORATORY. (THE RELEVANT ERRORS ARE LISTED UNDER THE HEADINGS STANDARD DEVIATION AND COEFFICIENT OF VARIATION: THE COLUMN HEADED PROBABLE MINIMUM ERROR SUGGESTS THE LOWEST LIMITS OF ERROR WHICH COULD BE ATTAINED BY ONE HIGHLY SKILLED OBSERVER)

Investigation	No. of observations	Mean	Standard deviation	Coefficient of variation (%)	Significant diff. $\pm 2\sqrt{2s^2}$	Range $\pm 2s$	Probable minimum error	Some previous estimates of error	Some previous estimates of the normal range
Haemoglobin, Haldane method	50	97	5.1%	5.2	14.4%	87–107%	2–3%	2–13% Macfarlane (1945)	84–116% Price-Jones et l. (1935)
Haemoblobin, neutral grey photometer	50	97%	2.9%	3.0	8.2%	91–103%	2–3%	4.3% Macfarlane et al. (1948)	
Red cell count	50	4.39 millions	0.415 millions	9.5	1.17 millions	3.56–5.22 millions	7.8%	7.8% Berkson et al. (1935, 1940) 1.4% Wintrobe (1946)	4.5–6.4 millions Price-Jones et al.
Haematocrit, Wintrobe's method	50	45.5%	0.4%	1.0	1.1%	44.7–46.3%	0.5%	0.5% Wintrobe (1934)	
Haematocrit, micro-method	20	45.4%	0.9%	2.0	2.54%	43.6–47.2%	0.5%	0.5% Miller (1938-9)	
Colour index, Haldane method	50	1.12	0.12	10.7	0.339	0.87–1.36	8.10%		0.85–1.15 Osgood (1935)
Colour index, neutral grey photometer	50	1.12	0.11	9.9	0.311	0.90–1.34	8–10%		
Mean corpuscular haemoglobin concentration, Haldane method	50	31.44%	1.59%	5.0	4.49%	28.26–34.62%	2–3%	2.08% Wintrobe (1931-2)	28.17–38% (Various authors)
Mean corpuscular haemoglobin concentration, neutral grey photometer	50	31.44%	0.97%	3.1	2.74%	29.5–33.4%	2–3%		
Mean corpuscular volume	50	104.6cµ	10.1cµ	9.6	28.6cµ	84.4–124.8cµ	7–9%	1.6% Wintrobe (1931-2)	

TABLE I (continued)

THE ERRORS OF SOME HAEMATOLOGICAL METHODS AS THEY ARE USED IN THE ROUTINE LABORATORY. (THE RELEVANT ERRORS ARE LISTED UNDER THE HEADING; STANDARD DEVIATION AND COEFFICIENT OF VARIATION; THE COLUMN HEADED PROBABLE MINIMUM ERROR SUGGESTS THE LOWEST LIMITS OF ERROR WHICH COULD BE ATTAINED BY ONE HIGHLY SKILLED OBSERVER)

Investigation	No. of observations	Mean	Standard deviation	Coefficient of variation (%)	Significant diff. $\pm 2\sqrt{2s^2}$	Range $\pm 2s$	Probable minimum error	Some previous estimates of error	Some previous estimates of the normal range
Mean cell diameter measuring 100 cells on one slide	25	7.04μ	0.231μ	3.3	0.65μ	6.58-7.50	0.07μ	0.073μ Price-Jones (1933) 0.072μ Mogensen (1938)	6.815-7.492μ Price-Jones (1933)
Mean cell diameter measuring 500 cells on one slide	Calculated	7.04μ	0.229μ	3.25	0.647μ	6.58-7.50	0.07μ		
Mean cell diameter, 5 observers measuring 100 cells on five slides (500 cells in all)	Calculated	7.04μ	0.09μ	1.28	0.254μ	6.86-7.22			
Reticulocytes, dry slide method	18	6%	2.4%	40	6.8%	1.2-10.8%		0.54% with 15% reticulocytes 0.44% with 2% reticulocytes Plum (1942)	
Reticulocytes, coverslip method	18	7.2%	3.1%	43	8.7%	10-13.4%			
Platelets, Dameshek's method	25	365 thousands	151 thousands	41	427 thousands	63-667 thousands	10-15%	3.8% Dameshek (1932)	
Platelets, Leimpert's method	25	246 thousands	56 thousands	23	158 thousands	134-358 thousands	10-15%	1.8% Olef (1935)	
Whole blood coagulation, Lee and White's method	40	4.8 min.	0.226 min.	4.7	0.64	4.35-5.25 min.			
Whole blood coagulation, Dale and Laidlaw's method	40	1.5 min.	0.228 min.	15.2	0.63 min.	1.04-1.96 min.			
Whole blood coagulation, capillary tube method	40	2.0 min.	0.276 min.	13.8	0.78 min.	1.45-2.55 min.			

eresyl blue, are counted with an oil immersion objective in relation to 1,000 red cells and the total number of platelets are assessed from a separate red cell count.

2. Lempert's (1935) modification of Kristenson's method. In this method the platelets are counted directly in a counting chamber after dilution to 1/20 with a freshly prepared solution containing brilliant eresyl blue to render the platelets more readily visible, and urea to haemolyse the red cells.

Percentage of reticulocytes.—The counts were made by two methods :

1. A drop of a saturated solution of brilliant eresyl blue in alcohol was allowed to dry on a perfectly clean slide, and a small drop of capillary blood collected on a clean coverslip was inverted over the dye to form a very thin film. The preparations were allowed to stand for half an hour for staining to occur.

2. Five drops of a mixture of one part of a saturated solution of brilliant cresyl blue in alcohol, and 4 parts 1.0 per cent sodium citrate in normal saline were placed in a small tube. To this was added one drop of blood. If these proportions are carefully observed the red cells will not crenate. The tubes were shaken and allowed to stand for twenty minutes at 37° C. The contents of the tubes were then thoroughly mixed, and films made on clean slides were stained with Leishman's stain. In both methods the reticulocytes were clearly visible, but in the coverslip method it was not always easy to prepare films in which the red cells were sufficiently separated for them to be counted with accuracy.

Whole blood coagulation.—This was measured by three methods :

1. Lee and White (1913). Venous blood was collected in a paraffined syringe and 1 ml. placed in each of four small test tubes of uniform bore which were kept at 37° C. The coagulation time was measured from the time of filling the syringe.

2. Dale and Laidlaw (1911–12). Small tubes of wide capillary bore, each containing a freely mobile lead shot, were prepared. These were rapidly filled with capillary blood and, held by forceps, were immersed in water at 37° C. The tube was inverted at intervals and the time at which the lead shot failed to move was recorded.

3. Capillary blood was taken into a number of fine capillary tubes, the ends of which were then sealed with plasticine. The tubes were kept at 37° C. Pieces of tubing were broken off at intervals until a clot was withdrawn (Sabrazes, 1904).

Red-cell fragility.—This was measured by a method similar to that of Dacie and Vaughan (1938). The saline dilutions differing by 0.02 per cent were made up individually from sodium chloride dried to a constant weight, and each was tested with a standard silver nitrate solution. Heparin was used as anticoagulant, the volume of plasma was adjusted to a normal haematocrit reading, and the blood was oxygenated immediately before use. In the original technique the blood was added to 1 ml. of each saline dilution from a dropping pipette ; in this experiment 0.02 ml. amounts were measured with a series of haemoglobin pipettes. After the tubes had stood in the refrigerator the degree of haemolysis was read against a set of dilutions from completely haemolysed blood made from each sample tested.

Results

The total errors for the methods studied, in terms of their standard deviations and coefficients of variations, are shown in Table I.

The experiments were planned to separate by analysis of variance those components of error likely to be of importance in routine practice. Thus, for example in the red cell count, errors due to pipettes and counting-chambers and to differences between observers could be calculated. In whole-blood coagulation on the other hand, where observers are unlikely to disagree, differences between the order of taking the samples on one day and between samples taken on separate days were measured.

The component errors (S_1, S_2, S_3 . . .) are related to the total standard deviation (S_T) by the formula :

$$S_T{}^2 = S_1{}^2 + S_2{}^2 + S_3{}^2 \text{ (Formula 1)}$$

Any part of the total error not attributed to a specific factor was classed as random variation.

Haemoglobin.—The errors inherent in estimating haemoglobin have been worked out with considerable care by Macfarlane (1945) and Macfarlane and others (1948). Our results confirm these more detailed observations.

In this experiment five observers each made ten readings both by the Haldane method and with the neutral grey photometer, using ten pipettes and a single sample of oxalated blood. From an analysis of variance the errors referable to the observers and the calibration of the pipettes could be calculated. In addition the components of error due to reading the neutral grey photometer, the calibration, and the filling of the pipettes were determined in a series of separate experiments. The error of reading the photometer was measured by making twenty readings from one diluted sample of blood. The error caused by inaccuracy in filling the pipettes combined with the error of reading the photometer was estimated by filling one pipette ten times and reading the results on the photometer. The error due to calibration of pipettes together with those due to filling and to reading the photometer was assessed by filling ten different pipettes and reading the results on the photometer. From these observations the component errors could be calculated, using Formula (1).
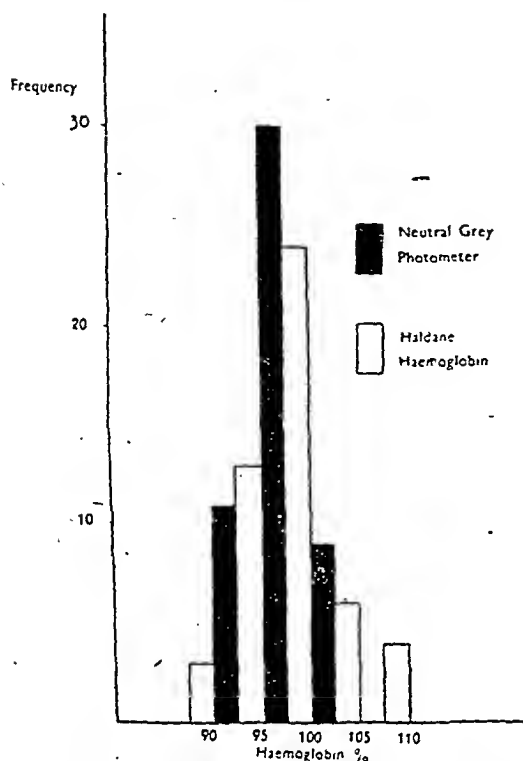
FIG. 1.—Histogram to show the distribution of individual readings for haemoglobin estimation by the Haldane method and using a neutral grey photometer. With each method five observers made ten estimations on one sample of venous blood using ten pipettes.

Fig. 1 shows the distribution of the different values for the haemoglobin obtained by the five observers. In the Haldane method there was a wider dispersion of the readings than was shown by the neutral-grey photometer. In Table II the errors for the two methods obtained by analysis of variance are compared with each other and with those derived from the separate experiments. It will be seen that the greater precision of the neutral-grey photometer results from a reduction in both random variation and the differences between observers.* In addition it will be seen that the random error of the analysis of variance for the photometer method, which necessarily includes that of filling pipettes, is entirely accounted for by this error, which was determined separately. Since the error of the observers and of reading the photometer are both of the order of 1 per cent, the error of the photometer method is probably

*Since only five observers took part in the majority of the experiments described, the observer component is seldom determined with great precision.

approaching the minimum for haemoglobin determination.

Red cell count.—The error of the red cell count has been the subject of much controversy. On the one hand Berkson and others (1935, 1940) have shown that the minimum error is of the order of 7.8 per cent, whereas other workers believe that far greater uniformity is possible. Wintrobe (1946) stated: " In spite of the fact that statistical analysis indicates that the error in counting red cells may be as great as 16 per cent even in the hands of trained technicians, it will be found that, with practice, counts agreeing within 200,000 cells per c.mm. can be made." Our results have favoured Berkson and others' estimate of the error, but it is also true that trained technicians can make counts that will agree within 200,000 cells. However, this uniformity cannot be maintained if several skilled workers each make a series of independent counts. It will then be found that their mean values differ more widely than would be expected from chance variation (Biggs and MacMillan, p. 288 of this issue). The " accuracy " of the skilled haematological technician is a product of the method of training. It is usual to set a standard of agreement between repeat counts which must be achieved before a worker's results are considered reliable. Since this standard is invariably one that cannot be reached by accurate

TABLE II

THE COMPONENTS OF ERROR IN HAEMOGLOBIN ESTIMATION EXPRESSED AS A PERCENTAGE OF THE MEAN HAEMOGLOBIN VALUES: BASED ON FIFTY OBSERVATIONS MADE BY FIVE OBSERVERS ON A SINGLE SAMPLE OF BLOOD

Source of error and mean value	Estimation of error by separate experiments (%)	Analysis of variance in the neutral grey photometer method (%)	Analysis of variance for Haldane method (%)
Mean ..	97	97	97
Total error ..	3.3*	3.0	5.2
Calibration of pipettes ..	2.0	1.8	·2.4
Filling pipettes	2.2		
Observers ..		1.0	2.8
Reading photometer ..	1.38		
Random error		2.2	3.7

* Excluding the observer component.

counting, the technician learns to count very rapidly and · to make continuous unconscious adjustment–in a series of counts to ensure that all will agree with the first.

In this experiment five observers made ten· red cell counts from the sample of blood used in the haemoglobin determinations, using ten pipettes and ten counting chambers. One red cell pipette .was always used with the same counting chamber, and thus the errors due to these two factors were estimated together.

It will be seen from Fig. 2 that the total range of variation was very large, rather larger than that

Frequency
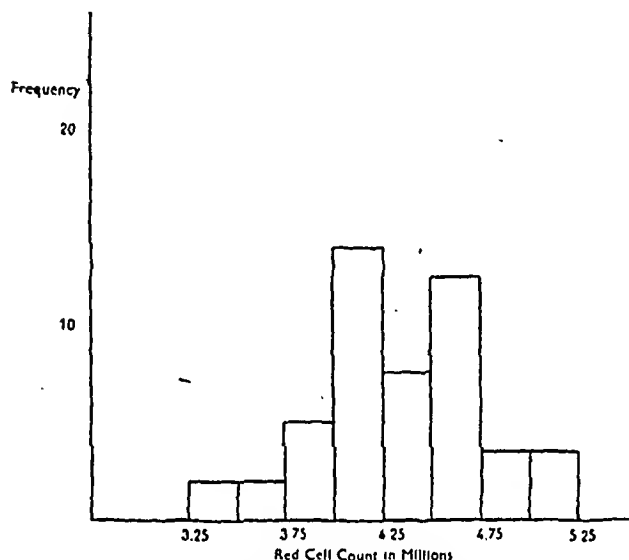
FIG. 2.—Histogram to show the distribution of individual red cell counts. Five observers each made ten estimations from the sample of blood from which the haemoglobin determinations were made, using ten pipettes and ten counting chambers. Each pipette was always used with the same counting chamber.

reeorded by Berkson and others. This increase in error is due both to the inclusion of different observers in the experiment and to the fact that, whereas Berkson and others counted the red cells with an eleetrie eounter from photographs, in these experiments the eells were counted by eye. In addition it will be seen from Table III that the component errors are rather different. Berkson and others reeord a larger error for pipettes and counting chambers, while our resu'ts show a greater random fluctuation. This diserepancy is due to the different methods used to diseover the errors. Berkson and others made a series of separate experiments similar to those described for the errors of the haemoglobin pipette and reading the neutral-grey photometer, while our results were

TABLE III

THE COMPONENTS OF ERROR IN RED CELL COUNTING EXPRESSED AS A PERCENTAGE OF THE MEAN VALUES FOR THE RED CELL COUNT. THE RESULTS OF OUR EXPERIMENTS COMPARED WITH THOSE OF BERKSON AND OTHERS (1940)

Source of error and mean value	Results from analysis of variance in our experiments	Results of Berkson and others' experiments
Mean value ..	4.39 millions per e. mm.	
Total error ··· ..	9.46%	· 7.8%*
Pipettes	4.26%	4.7%
Counting ehambers		4.6%
Observers	3.12%	- ·
Random error ..	7.8%	4.2%

* Excluding the observer component.

obtained by analysis of variance. The figure for random error in our calculations includes the error of filling the pipettes, which was estimated separately by Berkson and others.

The variability of this method, from which repeated counts on the same sample may be equivalent to differences of more than 1,000,000 cells per e.mm. in a 5,000,000 count indicates that small changes in red cell count cannot be measured · with reasonable precision.

Packed cell volume.—A previous experiment in which Wintrobe (1934) estimated the error of the haematoerit as 0.5 per cent suggests that this method should give uniform results.

In this experiment each of the five observers filled ten Wintrobe haematocrit tubes with blood from the sample used in the red eell counts and haemoglobin determinations. These were then centrifuged at 3,000 r.p.m. for half an hour. There were no differenees to be detected between the tubes. The only error was a small random fluctuation of 0.5 per cent, giving a coefficient of variation of 1 per cent. This method has an almost negligible error and since some of the teehniques applieable to capillary blood are also reliable (Miller, 1938–39) the method is well adapted to routine use. The error of a capillary method in use in this laboratory is shown in Table I.

Indices.—The red cell counts, the determinations of haemoglobin, and the packed eell volume were all made on the same sample of blood, and sinee the relations between the haemoglobin and
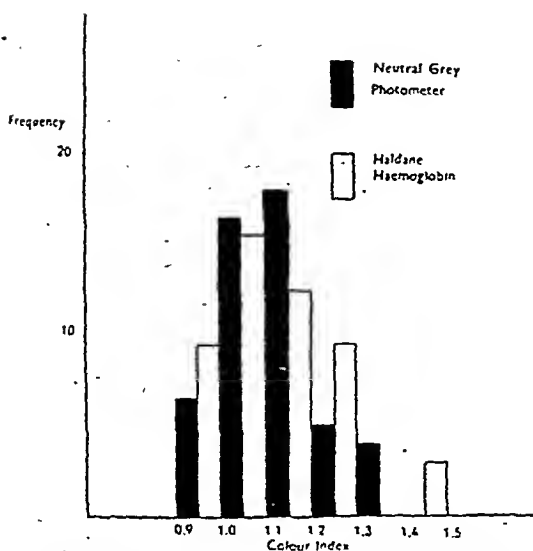
FIG. 3.—Histogram to show the distribution of the calculated values for the colour index using both the Haldane and neutral grey photometer methods. Each haemoglobin estimation was paired at random with a red cell count made by the same observer.

red cell count, the haematocrit and red cell count, and the haematocrit and haemoglobin for normal blood can all be represented by linear regression lines passing through the origin, the errors may be calculated with sufficient accuracy by pairing appropriate values at random and determining the standard deviations of the resulting indices.

The magnitudes of these errors are given in Table I, and the distribution of the individual observations in Figs. 3 to 5. It is obvious that the indices derived from variable observations are unlikely to measure small differences reliably, but the wide range of the colour index and mean corpuscular volume is illuminating. By chance the sample of blood examined gave mean values for the colour index and mean corpuscular volume which are at the upper limit of the normal range 1.12 and 104.6 μ respectively. It will be seen that in a blood sample of this type, encountered not infrequently in routine work, there is a high probability of recording a grossly pathological value. The fact that these exaggerated figures seldom reach the clinician may be

attributed to the natural prejudice of technicians and pathologists in favour of reasonable results; a prejudice which leads to the repetition of values incompatible with the appearance of the blood film or grossly different from previous observations on the same patient.

Both of these indices are used to measure differences that are small in relation to their range of chance fluctuation. Neither is therefore of great value as a single observation, though successive readings will give a general impression of any systematic trend over a period of time, such, for example, as may occur during the treatment of pernicious anaemia.

On the other hand the mean corpuscular haemoglobin concentrations showed relatively little variation, and this index, which is well adapted to the study of iron deficiency, could be used more frequently as a routine estimation.

Mean cell diameter derived from the Price-Jones curve.—Both Price-Jones (1933) and Mogensen (1938) have studied the errors inherent in drawing a Price-Jones curve, including the variation in cell size in one subject on repeated measurements from different slides, and the range in normal subjects (Price-Jones, 1933). The error in making repeated observations on one subject was small, but it is usually believed that the technique, in addition to being extremely time-consuming, is of little value except in the hands of highly skilled and experienced observers. Our experiment was undertaken to discover whether relatively unskilled workers could obtain information of any clinical value. Five observers measured a hundred cells from each of five slides taken from the same subject at the same time. The errors referable to differences between observers and those between slides could therefore be calculated.

It will be seen in Fig. 6 that a wide variation occurred. The range of cell size in any one estimation (the standard deviation of one Price-Jones
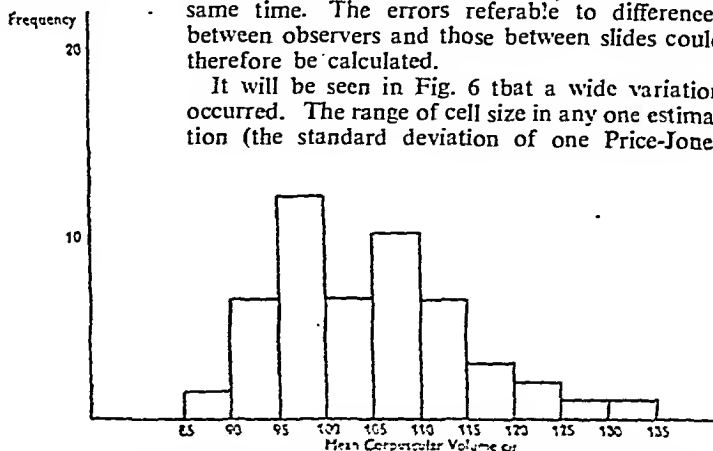
FIG. 4.—Histogram to show the distribution of the calculated values for the mean corpuscular volume. Each of the red cell counts was paired at random with a haematocrit reading made by the same observer.
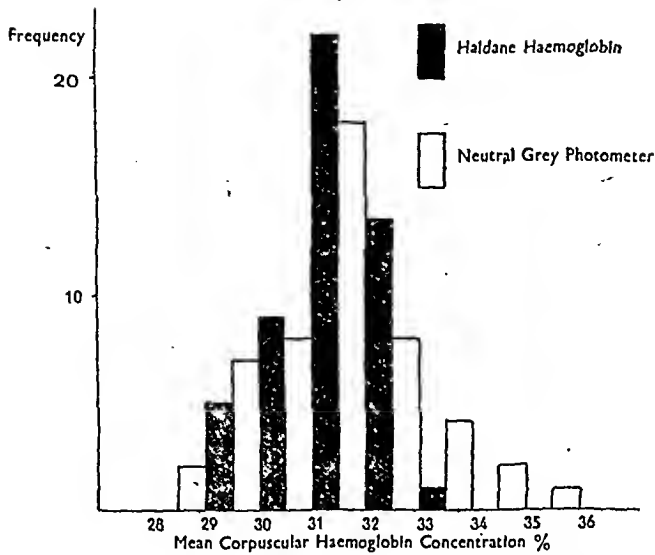
FIG. 5.—Histogram to show the distribution of the calculated values for the mean corpuscular haemoglobin concentration using both the Haldane and neutral grey photometer methods for haemoglobin determination. Each haemoglobin determination was paired at random with a haematocrit reading made by the same observer.

curve) was similar to those of Mogensen and Price-Jones and remained fairly constant throughout. The variation between different slides was also similar to those found by Mogensen and Price-Jones (Table IV). The increased error was due to differences between observers and a component classed as "interaction." Neither Mogensen nor Price-Jones considered the possibility that
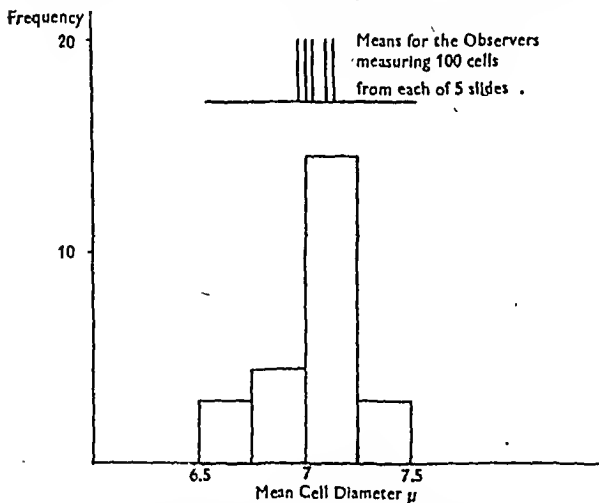
FIG. 6.—Histogram to show the distribution of individual determinations of the mean cell diameter. Five observers measured one hundred cells from each of five slides. The mean values for each observer measuring one hundred cells from the five different slides are shown above.

TABLE IV

THE COMPONENT ERRORS OF DETERMINING THE MEAN CELL DIAMETER FROM PRICE-JONES' CURVE EXPRESSED AS STANDARD DEVIATION IN μ. THE RESULTS OF OUR EXPERIMENTS COMPARED WITH THOSE OF MOGENSEN (1938) AND PRICE-JONES (1933)

Source of error and mean value	Error determined from our experiments	Error assessed by Mogensen	Error assessed by Price-Jones
Mean	7.04	7.44	7.328
Total error	0.231		
Error for 1 observer measuring 100 cells on one slide	0.143	0.0721	0.0735
Differences between observers	0.171		
Differences between slides	0.08	0.06	0.06
Error component classed as "interaction"	0.1265		
Random error per mean of 100 cells	0.044	0.045	0.048

observers might not always agree, a factor of importance in clinical practice.

As was suggested by Mogensen (1938), and more recently by Humble and Belyavin (1948), the only error reduced by increasing the number of cells measured is that due to chance variation in the population of cells selected from a particular slide. In our estimations this error was 0.04 μ which, compared with a total error of 0.231 μ, is negligible, and no appreciably greater precision results from increasing the number of cells measured to 500 (Table I). On the other hand, if the larger errors due to the slides and observers were reduced, the precision would be increased. This can be effected if, for example, five observers each measure a hundred cells from different slides. Since only five hundred cells are measured by the five observers together, there is no increase in the total labour of Price-Jones' original technique ; in this way the error is reduced from 0.231 to 0.09 μ. In Fig. 6 the mean values for the five observers' measurements on different slides are compared with the total invariability. Although the error of the mean cell diameter is reduced by this method, a false impression of anisocytosis will follow

systematic differences between observers. This may be avoided if the Price-Jones curve is constructed from the results of one observer.

The error is large compared with those of Price-Jones and Mogensen, but it is small in comparison with those of other investigations that give any indication of cell size (Mean Corpuscular Volume and Colour Index). In addition the Price-Jones curve gives a valuable record of anisocytosis. Again, if highly reproducible results are required, the method by which five observers make measurements on five slides will give a precision approaching that of the experts, even including the observer component. Thus from the view point of precision the Price-Jones curve, even when constructed by the average laboratory worker, will give information of clinical value. The main disadvantage that remains is the length of time occupied by a single observation. However, we did not find that this was excessive. In forty-five minutes one observer may make all necessary measurements and complete the calculations, using a simplified technique such as that described by Humble and Belyavin (1948); no doubt this time could be reduced still further. Thus, if, in a case of pernicious anaemia for example, instead of making repeated observations of the Colour Index and Mean Corpuscular Volume, two Price-Jones curves were drawn, one before and the other following treatment, there would be little total loss of time, and the record of the patient's progress would be more complete and more precisely assessed.

Reticulocyte count.—Plum (1942) and Jacobsen and others (1947) have shown that repeated reticulocyte counts on one sample of blood will agree closely. It is, perhaps, difficult to understand why the errors which they record appear rather smaller than the random selection of 1,000 cells from a binomial distribution would allow. Nevertheless, even acknowledging the full standard deviation to be expected, which is of the order of 1 per cent of reticulocytes for counts from 5 to 10 per cent, it might be hoped that this would prove one of the more reliable routine methods.

In this experiment nine observers counted the number of reticulocytes in five hundred red cells from one sample of venous blood. They made two preparations by each of the methods, and thus made four counts in all. In this way any difference between the observers and between the two methods could be measured. No consistent difference between the methods was found.

From Fig. 7a it will be seen that a large range of variation occurred, very much larger than should result from random selection from a binominal distribution. In both methods there was a considerable difference between the results of different observers (Table V). In the coverslip method there was also a big random error, probably caused by difficulty in accurate counting on films in which the cells were not well separated. In the slide method the two counts made by each observer agreed fairly well and thus the random error was small. Of these two, the slide method was therefore preferable.

The large observer error was, however, surprising, and an experiment was designed to decide whether this was due to differences between the preparations made by the observers or to difficulty in the recognition of reticulocytes. Six experienced
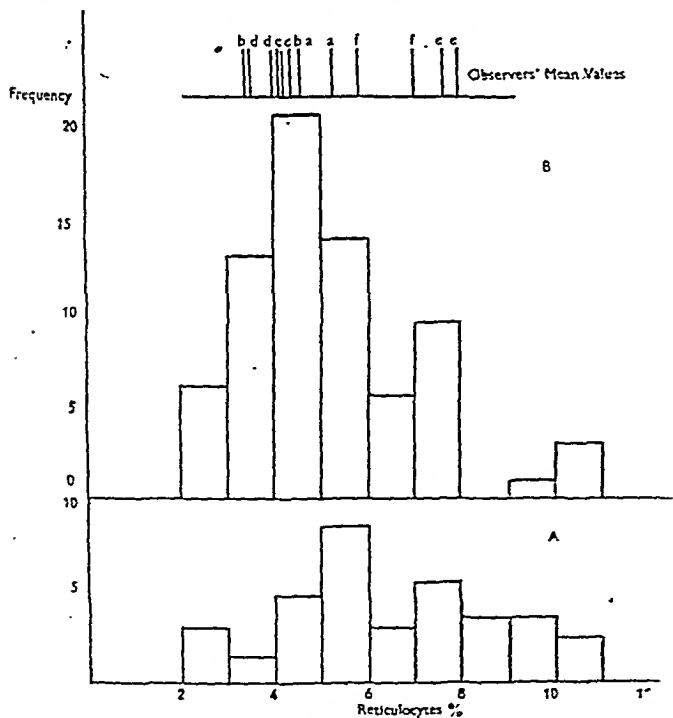
FIG. 7.—Histograms to show the distributions of the individual reticulocyte counts. The two parts of the diagram refer to different experiments (see text). In Fig. 7b the mean values obtained by the observers by the two methods of counting are indicated.

TABLE V

THE COMPONENT ERRORS OF THE RETICULOCYTE COUNT EXPRESSED AS STANDARD DEVIATION IN PERCENTAGE OF RETICULOCYTE

Source of error and mean value	Coverslip method (%)	Dry slide method (%)
Mean	7.2	6.0
Total standard deviation	3.1	2.4
Error due to observers	2.2	2.2
Random error ..	2.2	0.9

observers made counts from six slides made at the same time from one stained drop of blood. Inaccuracy in enumeration was avoided by reducing the field to contain between ten and thirty cells. The errors due to uneven distribution on one slide was minimized by making counts not only by recording the number of reticulocytes in five hundred red cells but also by a method of inverse sampling suggested by Woolf (1948). A hundred reticulocytes were counted and the number of fields passed over before this total was reached was recorded. The number of red cells per field was calculated from a random sample of 200–300 red cells. In this way the number of reticulocytes in approximately 2,000 red cells was assessed.

From Fig. 7b it will be seen that there was slightly more tendency for the values to be grouped than in the original experiment, but the variation was still large. The individual observers obtained fairly consistent results with figures ranging from 2.4 to 4.8 per cent, 3.2 to 4.2 per cent, 2.6 to 6.4 per cent, 4.1 to 6.6 per cent, 4.4 to 7.2 per cent, and 6.4 to 9.8 per cent, but clearly the divergence of opinion between them was wide. A further study of this observer difference has shown that the number of reticulocytes seen is controlled both by the eyesight of the observer and by the magnification of the microscope used. Since this is an error that cannot readily be eliminated, the best results for clinical purposes will be obtained if the same observer makes all the counts on any one patient.

Platelet count.—The indirect and direct platelet counts, of which Dameshek's and Lempert's methods were chosen, are known to give different figures for the normal number of platelets. The indirect methods, in which the platelets are counted in proportion to the number of red cells and the total deduced from a separate red cell count, give the higher results, and therefore have the advantage of preserving a greater proportion of the platelets. From the techniques involved neither of these methods can be precise. In Lempert's method the error must include those due to pipetting and the uneven distribution of platelets. The minimum error of this method is likely to exceed that of the red cell count because fewer particles are counted. In the indirect methods the error cannot be reduced below that of counting red cells, combined with the error due to uneven distribution of the platelets on the slide preparations. In addition to these the scope for divergence of opinion between observers is great. According to Aggeler and others (1946), ". . . there may be wide variations in the results obtained by different observers, each using the same method of counting. The individual differences appear to depend upon the microscopic technique and visual acuity of the observer. We are convinced that it is impossible to standardize the platelet count so that the results of even expert technicians will not show significant variation." An approximate minimum error might be expected to lie between 10 and 15 per cent, and including an observer component the error might well be considerably greater. It is therefore rather surprising that previous estimates of error are extraordinarily small; that calculated from Ivanitsky-Vasilenko and Klimova's (1937) results in the Lempert method gives a random error of 0.2 per cent, Dameshek's figures (1932) give an error of 3.8 per cent, and Olef (1935), using an indirect method,
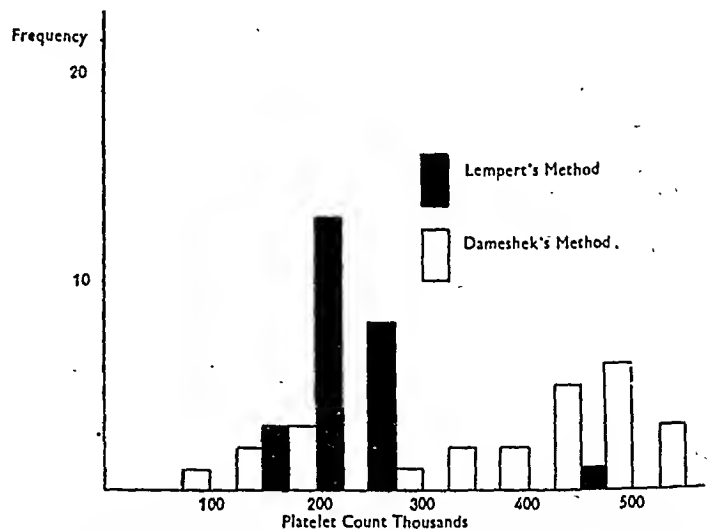
FIG. 8.—Histogram to show the distribution of the individual platelet counts by Dameshek's and Lempert's methods.

gives figures with a coefficient of random variation of 1.8 per cent. It would seem probable that unconsc ous bias must have been responsible for these very low figures.

In this experiment five observers each made five platelet counts using five pipettes and counting-chamber combinations. The counts were taken at

TABLE VI

THE COMPONENT ERRORS IN COUNTING PLATELETS EXPRESSED AS A PERCENTAGE OF THE MEAN VALUES OBTAINED BY THE TWO METHODS: THE ERRORS FOR PIPETTES AND ORDER OF TAKING THE SAMPLES ARE OMITTED BECAUSE THEY ARE SMALL IN RELATION TO THE RANDOM ERROR

Source of error and mean value	Dameshek's method	Lempert's method
Mean	365,000 c.mm.	246,000 c.mm.
Total coefficient of variation	41%	23%
Error due to observers	33%	13%
Random error ..	22%	17%
Random error (excluding one reading which differed markedly from the majority of observations) ..		6%

hourly intervals from a different finger of the same subject. Thus the errors due to observers, calibration of pipettes, and counting chambers, and the time of taking the sample could all be calculated.

The distribution of the individual counts are shown in Fig. 8. Lempert's direct method shows far less variation than does the indirect method of Dameshek, and if one observation which differed markedly from the rest is excluded the random variation approaches that due to the Poisson distribution. It will be seen from Table VI that the superiority of Lempert's method follows a decrease in the difference between the counts of the five observers. In both these methods the errors attributable to the pipettes and time of taking the samples were small in comparison with those of the observers and the random variation.

These results therefore reveal errors well above the probable minimum for the method, an error which could certainly be reduced if one experienced observer made a series of counts. However, for clinical purposes great accuracy is not required and the results obtained by Lempert's method are adequate.

Whole-blood coagulation.—Pauwen and others (1942) studied the coagulation time of normal venous blood. They found that the coagulation time of different normal subjects measured as an average of four tests did not show any very great variation, but that the four tests taken at one time might differ widely. Their figures show a random variation of about 15 per cent.

In this experiment four observations of the whole blood coagulation were made by each of the three methods on one subject on ten consecutive days. In the Lee and White method the order in which the tubes were filled with blood was recorded. In the Dale and Laidlaw method the four observations were made consecutively, on the same finger, a second prick often being necessary. The order of the tests was recorded. In the capillary tubing method all the tubes were filled from the same prick. In this way differences between the methods, between the order of taking the samples, and between samples taken on different days could be estimated.

The different methods are known to give distinct values for the coagulation time. From Fig. 9 it
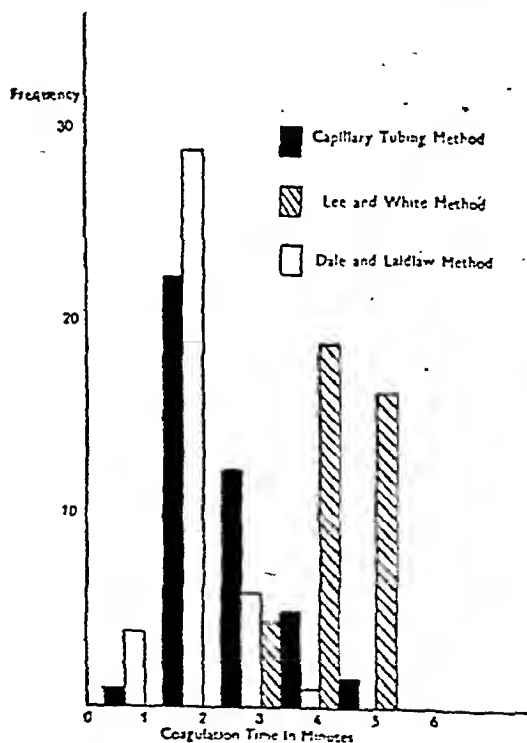
FIG. 9.—Histogram to show the distribution of individual determinations of the whole blood coagulation time by three methods.

will be seen that the individual readings for the Lee and White and the Dale and Laidlaw methods showed approximately the same dispersion about their means, but since the mean value for the Dale and Laidlaw method is lower the error is relatively larger (Table VII). In the capillary tubing method

TABLE VII

THE COMPONENT ERRORS IN THE MEASUREMENT OF BLOOD COAGULATION EXPRESSED AS A PERCENTAGE OF THE MEAN VALUES

Source of error and mean value	Lee and White method	Dale and Laidlaw method	Capillary tubing method
Mean	4.8 min.	1.5 min.	2.0 min.
Total coefficient of variation for tests made over a period of 10 days ..	10.8%	27.5%	29.4%
Total coefficient of variation for tests made on one day	4.7%	15.5%	14.0%
Error due to the order of taking samples on one day	6.2%	20.9%	0
Error due to variations between the different days on which samples were taken	9.7%	22.5%	25.9%
Random error ..	7.0%	25.1%	28.0%

the variation was greater. From an analysis of the constituent errors it was seen that both the methods using capillary blood showed a greater change from day to day than did the method of Lee and White. The Dale and Laidlaw method also showed a big discrepancy between the tests taken at one time, and from Table VIII it will be seen that the first test had a longer coagulation time. From the method of taking the samples this was probably due to a larger amount of tissue juice in tests after the first. In the Lee and White method the first tube often coagulated more quickly than the others, and presumably thromboplastin from the needle was included in this specimen.

These results illustrate the small variability of the coagulation time as tested by the Lee and White method. The technique appears to be more reliable than that of Pauwen and others (1942).

Red cell fragility.—Many of the factors which influence the red cell fragility curve, including the degree of anaemia, oxygenation of the blood, the pH of the saline solutions, and the temperature of incubation, have been worked out by Dacie and Vaughan (1938). They used a technique in which all these factors were considered to determine the red cell fragility in fifty normal subjects, and thus set limits to the normal range. Since discrepancies between the results of different observers have arisen in relatively simple methods, it seemed possible that the red cell fragility curve, as used in a routine laboratory, might show a wider range of variation in normal people than was recorded by the originators of the technique.

TABLE VIII

A COMPARISON OF THE MEAN VALUES FOR FOUR CONSECUTIVE ESTIMATIONS OF BLOOD COAGULATION MADE BY THREE DIFFERENT METHODS (THE FIGURES GIVEN ARE THE AVERAGES OF THE VALUES OBTAINED ON TEN DIFFERENT DAYS)

Method	Order of test			
	1	2	3	4
Lee and White	4.4 min.	4.8 min.	5.1 min.	5.0 min.
Dale and Laidlaw	2.04 min.	1.38 min.	1.32 min.	1.40 min.
Capillary tubing	1.92 min.	2.12 min.	2.05 min.	2.10 min.

In this experiment, therefore, five observers measured the red cell fragility in fifty normal subjects. Ten observations were made by each of three observers from one laboratory, and twenty by two observers from a second laboratory. The same saline solutions were used throughout. In most cases duplicate tubes were set up to reduce the errors of pipetting. In addition the red cell fragility of one normal subject was tested on several different days.

The mean value for the median corpuscular fragility, 0.392 per cent, was higher than that of Dacie and Vaughan (1938), 0.366 per cent, because less blood was used in proportion to the volume of saline. The readings of the five observers, considered separately, showed variation in relation to their respective mean values which was comparable to that of Dacie and Vaughan (1938), but, since the observers' mean values were different, the total range was wider (Fig. 10). Thus, if the red cell fragility is always measured by one observer a range of variation comparable to that established by Dacie and Vaughan will include all the readings on normal people. On the other hand, if the test is made by a number of observers wider limits of normality must be set. The diversity of the red cell fragility curve in one normal subject
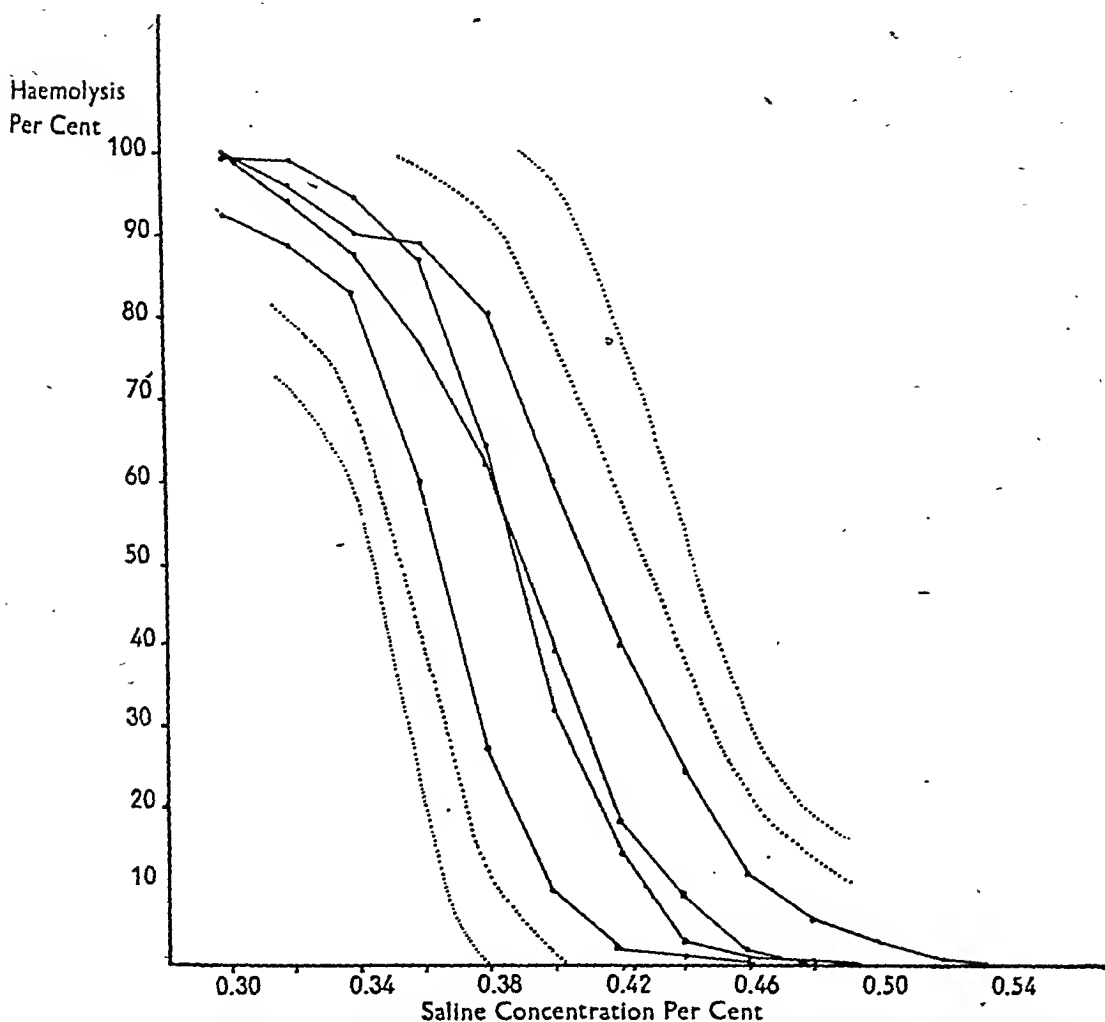
FIG. 10.—Diagram to show the mean values for the red cell fragility curve in normal people obtained by different observers (continuous lines). The limits of probable variation referred to in the text are shown by discontinuous lines.

(Fig. 11) also suggests that it would be unwise to accept a narrow concept of abnormality.

In Fig. 10 the mean values for the four sets of observations are given ; above and below these are indicated two limits of the probable variation in normal people. The narrower range represents one standard deviation above and below the means of the more extreme observers. These limits should include nearly 90 per cent of all the readings. The wider limits represent two standard deviations above and below the extreme observers, and these should include almost all normal fragility curves.

Discussion

In all these investigations emphasis has been placed on the use of a particular method as a routine procedure. We have therefore deliberately not required the observers to have specialized skill in any one technique. It may well be thought that the large errors obtained, in most cases larger than any previously recorded, are proof that none but those highly skilled can carry out these investigations adequately. To some extent this is true, for many of the techniques can be used with far greater precision. The Haldane method for haemoglobin estimation, for example, a notoriously

unreliable method in routine practice, can give very uniform results in the hands of one observer (Macfarlane, 1945). Again, several of the observers taking part in these experiments made reticulocyte counts on one sample of blood which showed very little variation, and Price-Jones (1933) and Mogensen (1938) have demonstrated the high precision of the Price-Jones curve. Though one skilled worker may obtain very uniform results it does not follow that the figures of different skilled observers will agree. In the Haldane haemoglobin estimation,

for example, Macfarlane (1945) has shown that the skilled observers almost invariably disagreed ; in this experiment the reticulocyte counts of the different observers varied, and a similar observation was made by Aggeler and others (1946) in counting platelets.

From this it is clear that, were it possible for one observer to carry out all the investigations, the variability of the results should approximate to the minimum error of the method. The probable minimum errors of some of the methods discussed
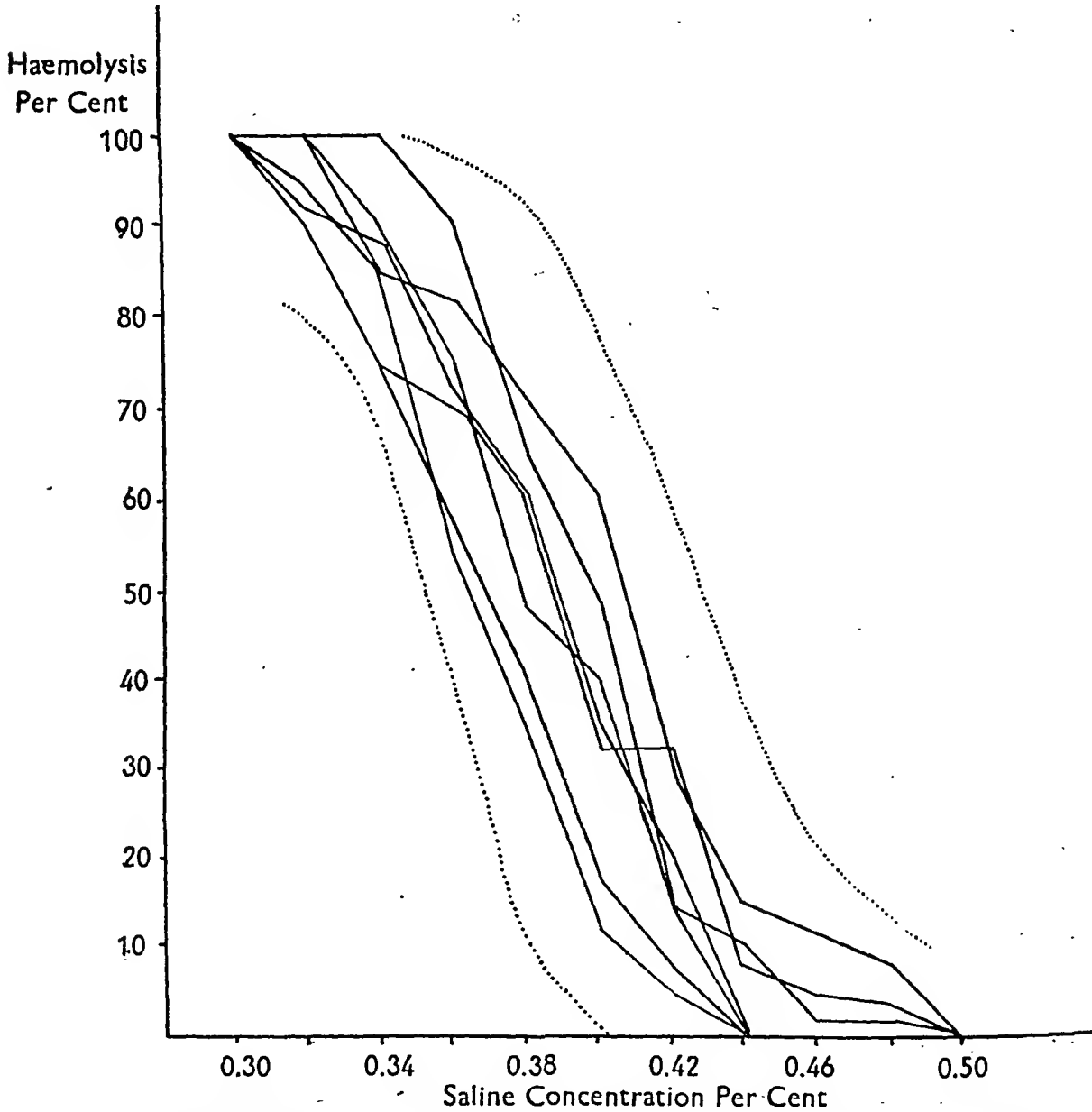
FIG. 11.—Diagram to show the range of variation in the red cell fragility curve in one normal subject.

EXTENT OF ERROR: PER CENT HAEMOGLOBIN
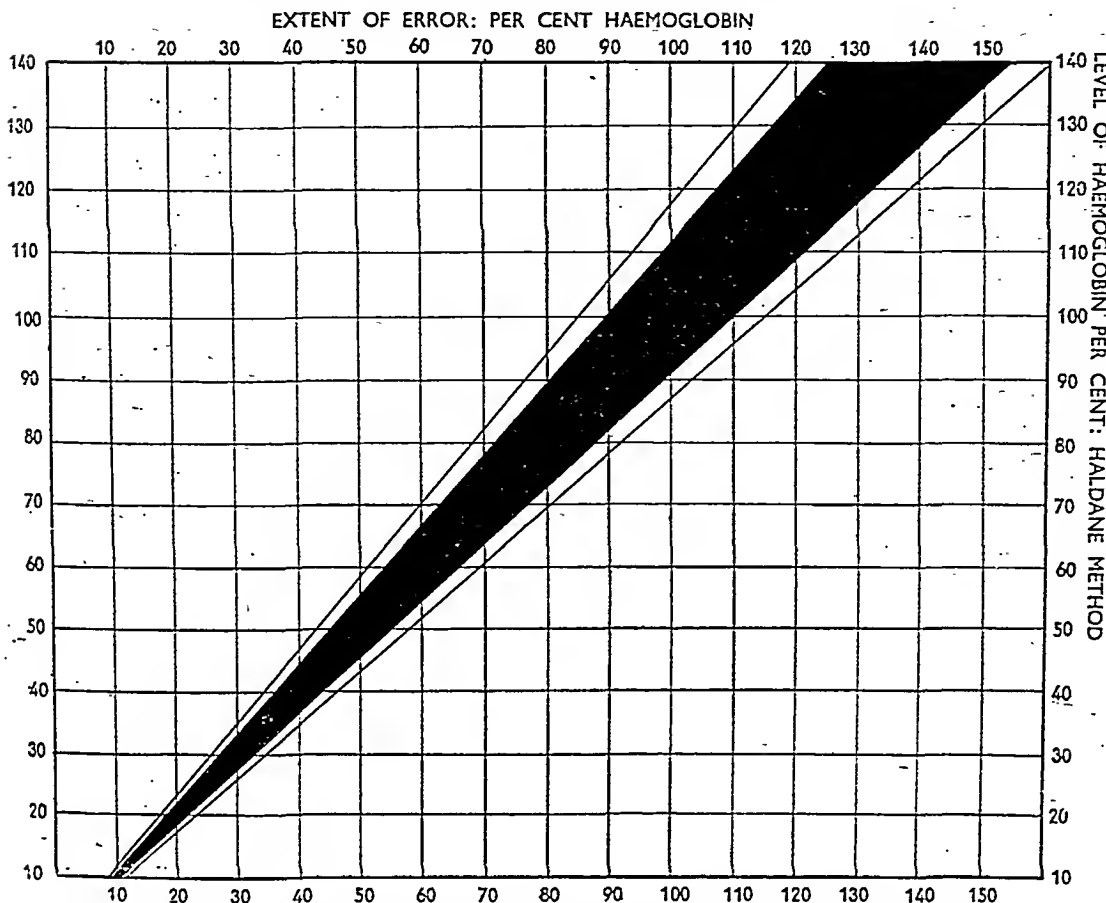
FIG. 12.—Diagram to show the error of haemoglobin determination by the Haldane method at levels of haemoglobin from 10 to 140 per cent. On the vertical axes the magnitude of the error is indicated. The solid area represents the range within which nineteen out of twenty repeated estimations on one sample will fall. The outer lines give the upper and lower limits that might be expected to contain nineteen out of twenty pairs of estimations on the same blood sample. In any two estimations on the same patient, therefore, a difference greater than these limits would have to be obtained before any significant change in haemoglobin could be inferred. The value is referred to as "the significant difference." The values for significant difference in Figs. 12, 13, and 14 are approximations because the error for an increase in the value is slightly different from that of a decrease. (The error should be calculated from $2\sqrt{\div S_2^2}$ for any given difference and is in fact calculated from $2\sqrt{s_1^2}$ at the upper level and $2\sqrt{s_2^2}$ at the lower level.)

Example of the Use of Fig. 12: If the haemoglobin is recorded as 70 per cent (vertical axis) repeated observations would be expected to lie between 63 and 76 per cent (horizontal axis). If the haemoglobin is 70 per cent on one occasion, a second observation must exceed 81 per cent or fall below 59 per cent before any significant change can be inferred.

are given in Table I. These limits are important, for it is common for skilled observers to improve on them. In the red cell count, for example, any skilled technician can, as Wintrobe has said. obtain results on one sample of blood that will agree within 200,000 cells. Again some of the recorded figures for platelet counting show the most extraordinary agreement (Olef. 1935 ; Ivanitsky-Vasilenko and Kilmova, 1937). This uniformity, which would be impossible for a mechanically impartial recorder, is the result of an unconscious bias which was clearly demonstrated by Macfarlane (1945) in the case of the Haldane haemoglobin determinations. Macfarlane showed that observers could use this method with a greater uniformity of results when provided with

graduated tubes than when only plain tubes were supplied. In the case of the red cell eount this excessive aceuracy can be shown to deerease, rather than inerease the reliability of the results (Biggs and MacMillan, 1948).

The question of importance is the eourse whieh should be followed in a routine laboratory. Investigations of whieh fifty to a hundred may be made in one day cannot all be made by one highly skilled observer. With these, it is essential to seleet those methods whieh give reasonably good results in the hands of the "average" observer. The methods of most importanee are those which assess the degree of anaemia and the response to treatment. The selection of the method for haemoglobin estimafion is elearly important and, as Maefarlane and others (1948) have shown, the methods with an error approaehing the minimum are the neutral grey photometer using oxyhaemo-globin, the Sahli-Zeiss comparator using acid-haematin, and the Duboscq photometer using various pigments. In addition, the haematocrit gives very reliable results and from these two observations the mean eorpuscular haemoglobin eoncentration may be caleulated. These measurements, together with the pathologist's opinion on the appearance of the blood film, give the best information available for routine diagnosis exeept in the macroeytic anaemias, where some assessment of cell size is necessary.

The mean eorpuseular volume and eolour index are at present routine procedures in all haematologieal laboratories, where their vagaries give rise to constant irritation. The labour involved by these measurements is, in our opinion, not justified by the results whieh they give. There are few pathologists who would aeeept the figures recorded by either of these indiees if they were not borne
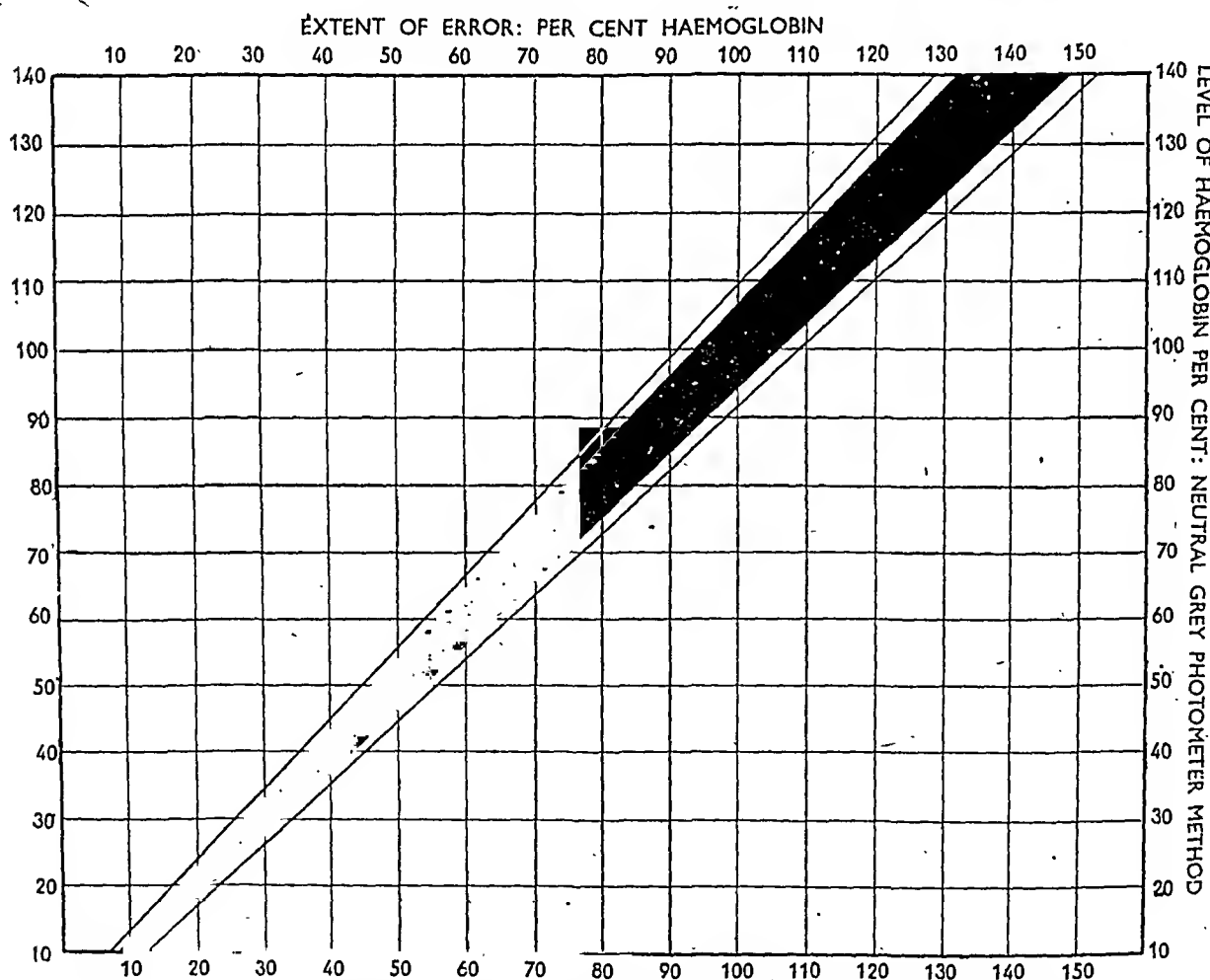
FIG. 13.—Diagram to show the error of haemoglobin determination by the neutral grey photometer method for levels of haemoglobin from 10 to 140 per cent (see legend to Fig. 12).

EXTENT OF ERROR: MILLION CELLS
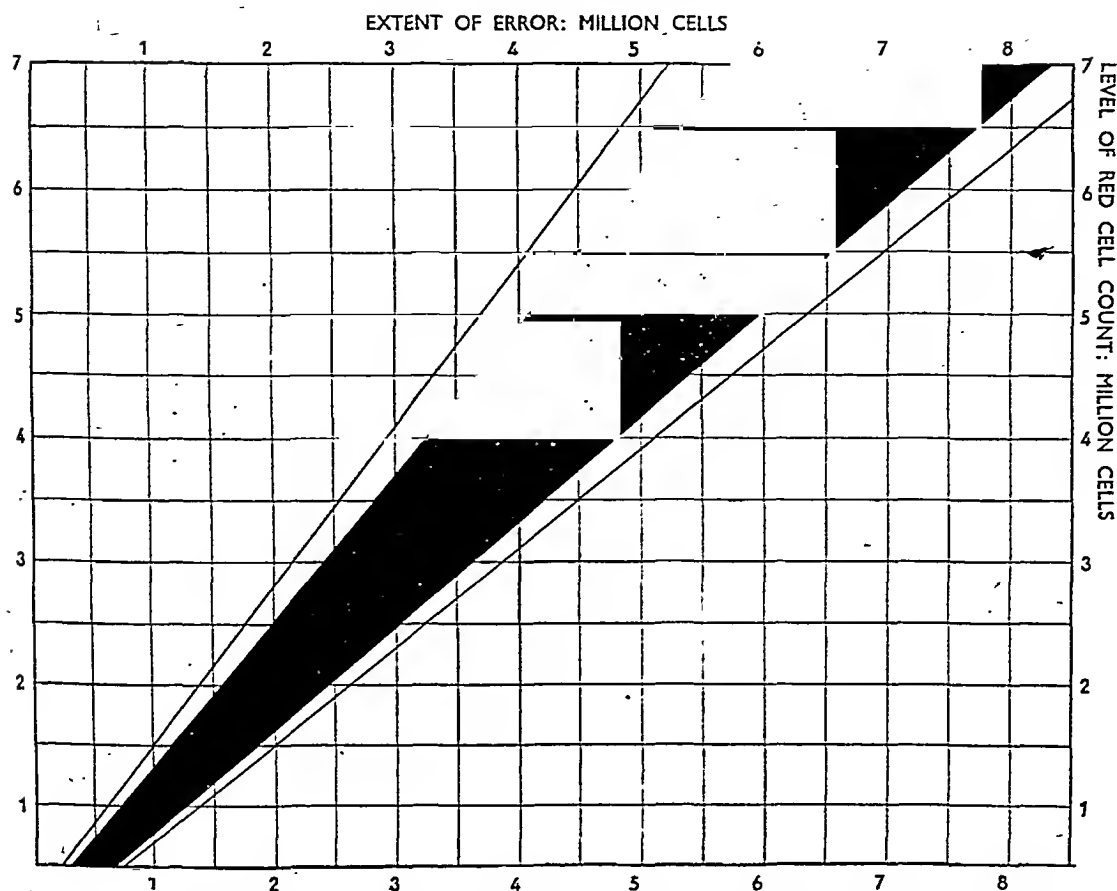
FIG. 14.—Diagram to show the error of the red cell count for counts varying from 50,000 to 7,000,000 using a 1/200 dilution of blood, and counting of eighty small squares in a Neubauer counting chamber (see. legend to Fig. 12).

out by the appearance of the blood film. This fact in itself illustrates a conscious or unconscious conviction that the figures are not reliable, a conviction well supported by these investigations. The only exception which must be made is in the case of macrocytic anaemia in laboratories where the drawing of a Price-Jones curve is difficult. In these cases repeated observations will give a broad general impression of the trend of cell size.

In the case of the reticulocyte count, where despite all care different skilled observers tend to disagree, the simple procedure of encouraging one observer to make all the counts on any one patient will increase the reliability of that patient's record.

In the case of investigations less frequently carried out, two possibilities exist. Single observers may be trained to attain specialized skill until their errors approach the minimum for the

method. Alternatively, the techniques may be carried out by a number of competent observers, when the range of their errors will be comparable to those recorded here. If the special investigation is complicated and the results require to be accurate, as for example in prothrombin determinations. the former procedure is preferable. On the other hand, many of the investigations need not be measured with very great precision for ordinary clinical purposes, and the results of the "average observer" are probably adequate. It would seem to us preferable to have a laboratory staffed by workers competent to carry out any investigation that could reasonably be requested, than a staff of specialists in single techniques.

The final problem is the application of these concepts of error to routine practice. The errors that we have calculated in most cases apply only

X

to the readings recorded. The error of haemo-globin determination by the Haldane method, for example, is relative to the level of haemoglobin. Thus an absolute error of 5.2 per cent at 100 per cent haemoglobin is reduced to 2.6 per cent at 50 per cent. The alteration in error with change in haemoglobin level is shown in Fig. 12. This diagram shows the range within which repeated observations on one sample may be expected to fall (solid area), and the difference between two haemoglobin readings which may be considered significant. In this method doubling the volume of blood used for low concentrations of haemo-globin would be expected to give little increased accuracy and must introduce additional error from the filling of pipettes on two occasions.

In the neutral grey photometer method the error of reading the photometer remains constant and though this error is small it becomes relatively more important at low levels of haemoglobin. Thus a slight reduction in error will result from doubling the amount of blood when the haemo-globin reading is less than 30 per cent. The errors of this method are shown in Fig. 13.

In the red cell count, as Berkson and others (1940) have shown, the error tends to increase with a decrease in the number of cells counted. Fig. 14 shows the errors that will occur with a constant technique of counting eighty small squares in a dilution of 1/200 using a Neubauer counting chamber. Some increase in precision will follow an increase in the number of cells counted. Using the technique outlined above, a count of one million cells will have an error of 12.6 per cent. By using a dilution of 1/100 and counting 160 small squares the error may be reduced to 9.5 per cent. Further reduction in error can be obtained by using two pipettes and two counting chambers. In the example given above the error would in this way be reduced to 7.8 per cent if a total of four hundred cells were counted (eighty small squares from each counting chamber using a dilution of 1/100).

The random error of the haematocrit is probably independent of the level of packed cells and thus will be proportionately greater at low haemotocrit readings. However, this error is sufficiently small to be negligible in comparison with the errors of other methods in use.

When reticulocyte counts are made by many different observers, the errors are so large that no useful limits of probable variation can be set. In the hands of one reasonably skilled observer the error approaches that of the binominal distribution. The limits of error for counts of five hundred and a thousand cells are shown in Table IX.

TABLE IX

THE ERROR IN ESTIMATING THE PERCENTAGE OF RETI-CULOCYTES WHEN ONE COMPETENT OBSERVER COUNTS 500 OR 1,000 RED CELLS WITH DIFFERENT PROPORTIONS OF RETICULOCYTES

Percentage of reticulocytes	Range within which the percentage of reticulocytes should fall is 19/20 re-peated counts on one sample		Significant differ-ence in percentage of reticulocytes	
	500 cells*	1000 cells†	500 cells*	1000 cells*
1	0–2	0–2	1.5	1.5
2	1–3	1–3	2.0	1.5
3	1–5	2–4	2.0	1.5
4	2–6	3–5	2.5	2.0
5	3–7	4–7	3.0	2.0
6	4–8	5–8	3.0	2.0
7	5–9	6–9	3.0	2.5
8	6–10	6–10	3.5	2.5
9	6–12	7–11	3.5	2.5
10	7–13	8–12	4.0	2.5
15	12–18	13–17	4.0	3.0
20	17–23	18–23	5.0	3.5
25	21–29	22–28	5.5	4.0
30	26–34	27–33	6.0	4.0
35	31–39	32–38	6.0	4.5
40	36–44	37–43	6.0	4.5
45	41–49	42–48	6.5	4.5
50	46–54	47–53	6.5	4.5

* Calculated from the standard deviation.
† From Snedecor (1946).

In platelet counting the error is so large that only conspicuous and progressive changes should be considered significant.

Summary

1. A series of experiments was devised to assess the errors inherent in the routine use of some common haematological methods. The results are summarized in Table I and in the figures illustra-ting the text.

2. Some of the sources of error were disclosed, and among these disagreement between observers was important.

3. An increase in the reliability of routine work will follow the selection of those methods which give the best results in the hands of the average laboratory worker.

4. In the diagnosis of anaemia the most precise measurements found in this investigation were: haemoglobin determination using oxyhaemoglobin and a neutral grey photometer, the haematocrit, the mean corpuscular haemoglobin concentration, and the mean cell diameter derived from the Price-Jones curve.

5. The red cell count has an error of 9 per cent and thus the colour index and mean corpuscular volume are too variable to record the small differences of interest to the clinician. The routine use of these indices might be restricted to the study of macrocytic anaemia in laboratories where a Price-Jones apparatus is not available.

6. The most important error in counting reticulocytes arises from disagreement between observers; this will be minimized if the same laboratory worker makes all the counts on any one patient.

7. The platelet count by both Dameshek's and Lempert's methods showed a large standard deviation, but Lempert's method was preferable.

8. Five observers recorded different mean values for the red cell fragility in normal people. In the routine laboratory it would be wise to use a wider concept of normality than that established by Dacie and Vaughan (1938).

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THE ERROR OF THE RED CELL COUNT

BY

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The staffs of haematological laboratories spend much of their time counting red cells. The precision of this estimation is therefore of practical importance, and many experiments have been designed to establish the error of the technique. In spite of this, the most widely divergent views are still held as to the reliability of single observations.

In 1881 Lyon and Thoma showed that the standard error of counts made on the same sample of blood was roughly proportional to the square root of the number of cells counted. Thus in a count of 100 cells the standard error would be roughly $\sqrt{100}$, and repeat counts on the same sample might by chance vary from 80 to 120 cells, or, in counts of 500 cells, the standard error would be approximately $\sqrt{500}$ or 4.5 per cent. In 1906–7 " Student " confirmed and stated more exactly the findings of Lyon and Thoma when he showed that the scatter of cells in a haemocytometer followed the Poisson distribution, in which the standard error is equal to the square root of the mean for the distribution. Thus, if 500 counts are made on one sample of blood and the mean value for each is 100 cells, the standard error is $\sqrt{100}$ and the majority of counts would show a range from 80 to 120 cells. From these observations it appears that there is an irreducible error in making a red-cell count which is approximately proportional to the square root of the number of cells counted.

In 1935 and 1940 Berkson, Magath, and Hurn again demonstrated the Poisson distribution of the red cells in a haemocytometer and made a thorough analysis of the other errors inherent in this technique. They showed that in a normal count the standard error is 7.7 per cent. An error of this magnitude implies that two counts on a normal sample of blood may differ by up to 1,100,000 ($\pm \sqrt{2S^2}$). In 1947 Hynes showed that the distribution of cells varies from one end of the coverslip to the other, and unless there is a reasonable length of coverslip on either side of the ruled area the error may be larger.

Wintrobe (1946) on the other hand maintained that repeat counts on a single sample should agree to within 200,000 cells, implying a standard error of 1.4 per cent, and several workers have published results in which this error has been achieved or even bettered (Mayers, 1922; Smith, 1931; Wintrobe, 1934; Price-Jones and others, 1935). This error is considerably less than that due to the Poisson distribution alone. Wintrobe (1946) does not recognize this distribution because he holds that the number of cells enumerated in the five separate squares of a red-cell count should not differ by more than 18. A greater internal variation is thought to indicate incomplete mixing of the contents of the pipette, and according to this criterion counts showing a greater variability should be rejected. In the Poisson distribution, on the other hand, differences of up to 40 cells might occur by chance. Wintrobe's opinion is apparently shared by the many skilled workers who habitually apply this criterion of adequate mixing. In fact, it is probably true that in spite of Berkson's careful experiments and statistical analysis many believe that Wintrobe's assessment of the error is more nearly true.

Experiments

We have therefore made several experiments in an attempt to find the origin of this apparently irreconcilable discrepancy.

Experiment 1.—Two reliable and skilled technicians made ten counts on a single sample of normal blood, using five pipettes and five counting chambers. In all these experiments one pipette was always used with the same counting chamber. The cells in a volume of 1/10,000 c.mm. of blood in five large squares were counted. The values for each square were recorded

TABLE I

THE NUMBERS OF RED CELLS RECORDED FOR EACH OF THE COUNTS IN EXPERIMENT 1

Observer	Pipette									
	1		2		3		4		5	
1	503	481	467	498	496	496	494	497	496	492
2	481	481	489	482	482	477	480	478	465	458

separately and the workers were requested to apply Wintrobe's criterion for complete dispersion of the cells in the pipette ; that is, to reject counts showing a variation greater than 18 cells between the five squares. They were aware that they were dealing with only one sample of blood, and were encouraged to compare results. The figures obtained showed close agreement (Table I). The first technician's counts had a coefficient of variation of 1.9 per cent and those of the second 2.1 per cent. In this experiment the technicians separately obtained a precision closely approaching Wintrobe's requirements. There was, however, a fairly large difference between the two sets of results, one giving a mean value of 477 and the other of 492.

Experiment 2.—Five technicians made five counts on one sample of normal blood using five pipettes and five counting-chambers. The blood was divided into five fractions, which the technicians had no reason to believe were derived from the same person. They did not compare results, but were requested to use Wintrobe's criterion for complete mixture of the contents of the pipettes. From Table II it will be seen that these counts show far greater variation than those of the first experiment. The coefficient of variation is now 7.6 per cent.

Experiment 3.—Five doctors who, though competent in the technique, were not engaged in red-cell counting as part of their daily work, made ten counts on the sample of blood used in Experiment 2, using ten pipettes and counting-chambers. These workers inevitably made their counts more slowly than the technicians, and—choosing the squares at random— were unable to obtain a close agreement between the counts for the different squares ; a difference of up to 40 cells was allowed. The results show an even greater variation, and the standard error is 9.5 per cent (Table III).*

TABLE II

THE NUMBERS OF RED CELLS RECORDED FOR EACH OF THE COUNTS IN EXPERIMENT 2

Observer	Pipette				
	1	2	3	4	5
1	430	404	437	453	517
2	475	405	455	412	443
3	437	440	508	435	488
4	488	449	485	463	505
5	458	397	458	436	447

* That this error is greater than that recorded by Berkson may probably be due to two main factors: (1) Berkson used an electric counter and enumerated the cells from photographs, whereas in these experiments the cells were counted by eye. (2) Berkson's experiments were made by one observer, and our experiments show that an error of 3 per cent may be attributed to differences between observers.

TABLE III

THE NUMBERS OF RED CELLS RECORDED IN EACH OF THE COUNTS IN EXPERIMENT 3

Observer	Pipette									
	1	2	3	4	5	6	7	8	9	10
1	427	372	418	440	349	484	430	416	449	464
2	434	420	385	472	415	420	415	396	439	424
3	480	421	473	496	474	411	472	423	502	488
4	451	369	500	464	444	410	422	396	459	471
5	462	453	450	520	489	409	508	347	440	391

Discussion

These experiments show that the magnitude of the error depends entirely on the condition of the experiment. The main components of error from an analysis of variance are shown in Table IV.

TABLE IV

THE COMPONENTS OF ERROR DERIVED FROM AN ANALYSIS OF VARIANCE IN EXPERIMENTS 1, 2, AND 3

Experiment	Total error %		Error due to observers %	Error due to pipettes and counting chambers %	Random error %
	Separate observers	Both observers			
I	1.9 2.1	3.6	2.0	0	2.5
II	7.6		2.9	4.8	5.1
III	9.5		3.1	4.2	7.8

The striking feature is the progressive increase in random error. In experiment 1, which is similar to those described by Wintrobe (1934) and Price-Jones and others (1935), the error is small if the results obtained by the two technicians are considered separately. The random error is well below that of the Poisson distribution (4.5 per cent). There is no component of error referable to the calibration and filling of pipettes and calibration of counting chambers, factors well recognized as a source of error in haematological technique. Moreover, it can be shown statistically that there is less than a 1/100 probability of obtaining mean values as widely divergent as 477 and 492 from the same sample of blood when the counts for each observer are so uniform.

Since there are good reasons for suspecting that the standard error recorded in the first experiment is not a true estimate, and since workers not engaged in red-cell counting as a routine are unable to obtain any close agreement, it is perhaps of interest to examine the process of training which leads to the making of uniform counts. This has been described by Emerson (1921), who recommended his medical students to make repeated counts on one person's blood until they achieved an agreement of 200,000 cells. He says, " Some students attain this accuracy quickly. Some, however, repeat this daily counting for twenty or thirty or even more days before their work is satisfactory to themselves or to us. By this time

they have certainly learned wherein lies the error of their technique. It is of interest that the most careful ones sometimes make the greatest errors since they take too much time where speed is essential." It seems probable that a training of this sort leads to an unconscious bias in favour of agreement between counts, a bias that was well demonstrated in Experiment 1.

In Experiment 2 there was no reason to obtain any agreement between the five sub-samples of blood, but the values for the last four squares of each count were biased by that of the first square (because an agreement between the five squares of 18 cells or less was required). In the third experiment the full range of random variation was shown.

It seems probable that any artificial reduction in the true variability will lead to a decrease rather than an increase in precision ; nevertheless it is possible that a skilled observer trained to make uniform counts on a single sample of blood might be able to select from each counting chamber squares typical of the whole field, which would estimate the mean value more precisely than a random selection. Experience in many sampling problems, however, suggests that subjective selection of this kind is a frequent source of unconscious bias. To test this point a further experiment was made.

Experiment 4.—Five technicians made routine red cell counts on eighteen different samples of blood and recorded separately the figures for each square. The counting chambers were then taken and the cells in all

TABLE V

ONE OF THE 18 COUNTS MADE IN EXPERIMENT 4.
(In the top row are the separate counts for each of the five squares obtained by the technicians. The counts for the separate squares of the whole field are recorded below.)

Technician's counts	80	82	79	82	77
Counts for whole field	77	69	62	69	70
	64	94	77	71	67
	85	73	66	67	72
	62	89	65	72	77
	64	64	67	77	62

the squares in the ruled area were counted. The results of one such experiment are shown in Table V. The mean value per square obtained as an average for the whole field is clearly a better estimate of the true mean value than that derived from any five

squares. Nevertheless if the five squares were chosen at random their mean value should not differ significantly from that for all the squares. When the mean values for each selection of five squares obtained by the technicians are compared with the corresponding mean for the whole field, several of the eighteen samples show a large difference, and statistical tests show that the dispersion about the field means is significantly greater than would be expected for the means of random samples. In other words, on an average, a random selection of five squares would give a more trustworthy estimate of the field mean than would the set of five selected by one of these technicians; the insistence on agreement between counts for the five selected squares has achieved a bias in estimation rather than an increase in precision.

Conclusions

It is concluded that : (1) the training of workers who make routine red cell counts should be a training in accurate counting rather than a training in achieving agreement between replicate counts; (2) in making a red cell count a difference of up to 40 cells should be allowed between the five separate squares counted; (3) from the most reliable estimates the standard error of the red-cell count lies between 8 and 10 per cent.

We should like to thank Dr. R. G. Macfarlane for his continued interest and advice, Mr. Finney for assistance with the statistical analysis, and graduate assistants and technicians of the department of haematology for their patience and willing co-operation in carrying out the experiments.

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A FURTHER CASE OF ANTI-LUTHERAN IMMUNIZATION, WITH SOME STUDIES ON ITS CAPACITY FOR HUMAN SENSITIZATION

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The "Lutheran" antibody was first described by Callender, Race, and Paykoç (1945) in the serum of a patient who developed a number of antibodies in response to multiple transfusions of blood. Callender and Race (1946) showed that the red cells of 8 to 9 per cent of the English population were agglutinated by this antibody, that it was inherited as a Mendelian dominant, and that it was unrelated to the ABO, MN, P, or Rh blood groups.

This paper reports a second example of this antibody found during the investigation of a transfusion reaction, and some attempt is made to assess the immunizing capacity of the Lutheran antigen.

Case History

In February, 1946, a man (A. T.) aged 38 with cirrhosis of the liver was admitted to the Radcliffe Infirmary (Reg. No. 10771/46) for treatment of bleeding oesophageal varices. He had suffered from recurrent haematemeses since 1931, and had been transfused, latterly at about yearly intervals. In the two months before his admission to this hospital he had received four transfusions, most of which had been accompanied by rigors. On March 1, 1946, he was transfused with four pints of group O Rh-positive blood. A slight reaction was noted though not reported till later. On March 7 he received a further two pints of O Rh-positive blood; this transfusion was followed by marked jaundice and haemoglobinuria, and on investigation two days later his blood group was found to be O Rh-negative, with no evidence of surviving Rh-positive cells, thus showing that he had destroyed all the blood transfused. His serum contained an incomplete anti-D antibody (titre 1:128 in concentrated albumin and 1:512 Coombs' titration), the obvious cause of his transfusion reaction. In addition there was an agglutinating antibody active at 20° and 37° C. This was also present at the same titre of 1:16 in the pre-transfusion sample taken on

March 1, 1946, which suggests that it had not caused the transfusion reaction. In fact all the bottles given had been cross-matched and found to be compatible and were later shown to be Lutheran-negative. As the serum also agglutinated the cells of a known Lutheran-positive donor, it was investigated in parallel with the original anti-Lutheran serum by Dr. S. T. Callender and Dr. R. R. Race. It agglutinated all of twelve Lutheran-positive and failed to agglutinate all of forty-seven Lutheran-negative bloods. The probability of such a coincidence being due to chance is infinitely small.

During April and May, 1946, the patient was injected with small amounts of Lutheran-positive cells. His subsequent transfusions were compatible both for the Rh and Lutheran factors. His antibody titres in June, 1946, were 1:512 incomplete anti-D (in albumin) and 1:16 anti-Lutheran. A year later the titre of the incomplete anti-D antibody had dropped to 1:64 and the anti-Lutheran antibody reacted weakly at a titre of 1:1. In January, 1948, no anti-Lutheran antibody was detectable in his serum.

The type of agglutination was similar to that previously described—compact agglutinates among many unagglutinated cells—and though with many of the test cells the serum gave slightly stronger reactions at 37° C., other cells were agglutinated more strongly at 20° C.

Serological Investigations

Three hundred and sixteen random bloods were tested with the serum; twenty-six were positive (8.23 per cent). It was possible to distinguish strong and weakly reacting types of positive cells; of the twenty-six Lutheran-positive cells fifteen were strong reactors or L_1 and eleven weak or L_2. When the serum (titre 1:16) was absorbed with an equal volume of packed cells the L_1 cells removed all the agglutinin, whereas when it was absorbed with the L_2 cells, which removed all antibody capable of reacting with themselves, it

TABLE

Name and Reg. No.	Recipients			Donors' Rh and Lutheran Group		Serum tested (days)	Immune investigations
	Age	Diagnosis	Rh Group				
E.W. 21754/44	46	Duodenal ulcer	R₀	R_1R_2	L +	15	1:16 Anti-Lutheran All previous
				R_1r	L +	25	1:4 „ - „ donors L —
						31	:2 „ „
A.P. 36929/44	46	Cholecystectomy	R₂	R_1R_2	L +	28	1:4 „ „ Previous donors·
				R_1R_2	L +	36	1:2 „ „ not available
						72	Negative
A.C. 45572/45	56	Anastomotic ulcer	R₁	R_1r	L +	11	Negative Previous donors
				R_0r	L +	17	Negative not available
C.B. 57191/46	12	Traumatic splenectomy	N.T.	R_1	L +	13	Negative
A.T. 54323/46	67	Partial gastrectomy	R_1r	R_1r	L +		
				$R''r$	L +	30	Negative
				R_1R_1	L +	68	Negative
W.M. 50740/46	59	Prostatectomy	R₂	rr	L +	20	Negative
P.M. 55258/46	32	Hysterectomy and salpingo-oophorectomy	rr	R_1r	L +	24	Negative
				R_1R_1	L +	58	Negative
						98	Negative
H. P.P.		Prostatectomy	N.T.	R_1r	L +	10	Negative
				N.T.	L —	42	Negative

N.T. = Not tested.

still agglutinated the more strongly positive bloods. This is analogous to the subdivision of the blood group A into A_1 and A_2 with the $α_1$ serum.

Cells

	L₁	L₂	L negative
Serum before absorption	++	+	—
Serum-absorbed L₁ cells	—	—	—
Serum-absorbed L₂ cells	+	—	—

This result cannot be explained by the dosage effect of homozygous and heterozygous bloods, as the expected frequency of Lutheran-positive homozygotes in 316 bloods would be less than one, whereas fifteen were observed. Five families have been studied, two of the L₁ and three of the L₂ type. In all cases the antigen was present in the same form in all members of the family. The evidence is in favour of there being three allelomorphs of the gene, their approximate gene

frequencies calculated from the 316 bloods being L₁ 2.5 per cent, L₂ 1.8 per cent, and 1 (Lutheran-negative) 95.7 per cent.

The occurrence of the same type of immune antibody in a second patient in the same ·hospital within two years suggests that the Lutheran factor is moderately antigenic in man, though this could not be accurately assessed, as both patients had developed other immune antibodies. The first case reported (Callender and others, 1945) was known to be hypersensitive to all blood antigens. It was therefore decided to transfuse Lutheran-negative patients with Lutheran-positive blood to determine the importance of the antigen in transfusion practice. Eight suitable patients were selected from those requiring transfusions, and their sera were examined for atypical antibodies. Each received without any reaction one or two pints of Lutheran-positive blood. Their sera were

re-examined at approximately fortnightly intervals for two months. Two out of the eight patients developed "Lutheran" antibodies of the agglutinating type, but no other antibodies, either agglutinating or incomplete, were detected in any of the eight patients, either on testing against a random panel of test cells or against the blood donors, who were recalled and whose cells were tested against the sera from the patients to whom their blood had been given. Both the patients who developed anti-Lutheran antibodies and one of those who did not respond had received earlier transfusions. In one of these (E. W.) all four donors of the earlier transfusions were tested and found to be Lutheran-negative; in the other two cases the donors were not traced. In both these deliberately immunized patients the anti-Lutheran antibody was detectable in the serum for a short time only. In the case of A. P. it was present at a titre of 1 : 4 twenty-eight days after transfusion, and it fell rapidly and was no longer evident forty-four days later. In E. W. the maximum titre of 1 : 16 was found fifteen days after transfusion; it fell to 1 : 2 during the succeeding fortnight.

Discussion

The chance finding of a further anti-Lutheran antibody and the immunization of two out of eight patients by transfusion with Lutheran-positive blood suggests that the Lutheran antigen is moderately antigenic in man and that supplies of suitable test serum could be obtained fairly readily. However, its antigenic capacity cannot be compared with most of the other blood factors antigenic in man, since very few systematic studies have been made. It had generally been considered that only 2 to 4 per cent of Rh-negative people were sensitized by transfusion of Rh-positive blood, but Hattersley (1947) has shown that eleven (55 per cent) of twenty Rh-negative Servicemen who had been previously transfused developed anti-Rh agglutinins. Diamond (1948) has confirmed this high incidence of immunization of Rh-negative subjects transfused with blood from unselected donors. In his series of over 500 cases, 46 per cent contained anti-Rh agglutinins. Other specific antibodies developing in response to transfusion have occasionally been reported. It is probable that they occur more readily than is generally recognized, as they are usually detected only in the investigations of a transfusion reaction or in the cross-matching of blood for a further transfusion.

Because of the rarity of the Lutheran antigen, its role as a potential cause of transfusion reactions is remote when unselected blood is used. In contrast to the anti-Rh agglutinin the serum titre of the anti-Lutheran antibody does not seem to be well maintained. In the case reported by Callender and Race (1946) the antibody was demonstrated in the serum for only about seven weeks, and though it was present in our first case for sixteen months after the first observation the titre had fallen from 1 : 16 to 1 : 4 in five weeks, subsequent reimmunization probably contributing to its persistence. In the experimentally immunized patients the titre was falling rapidly during the second month after transfusion. There was no indication of clinical incompatibility of transfusion in either of these recipients, though the donor cells must have been rapidly eliminated in case E. W., where an antibody titre of 1 : 16 was found fourteen days after transfusion; and though some changes in the peripheral haemoglobin levels were observed during this time these were not significant, as the patient was losing blood from a bleeding peptic ulcer.

Although the Lutheran antigen has not yet been proved to be the cause of a clinical transfusion incompatibility, it should, in view of its potential immunizing capacity, be considered when a transfusion reaction is being investigated.

Summary

A second example of human immunization to the Lutheran factor is described. In eight Lutheran-negative patients purposely transfused with Lutheran-positive blood, two developed anti-Lutheran antibodies. In none of the cases as yet described was there evidence of clinical incompatibility.

It has been found that there are strongly and weakly reacting Lutheran-positive cells, L_1 and L_2, analogous to the A_1 and A_2 groups. The importance of the Lutheran factor as a blood antigen is discussed.

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SOME OBSERVATIONS ON THIOURACIL NEUTROPENIA, WITH SPECIAL REFERENCE TO THE STERNAL MARROW

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It is generally agreed that leucopenia, with its clinical sequelae, is the most important and occasionally serious toxic manifestation in treatment with the widely used thiourea group of drugs (Lesses and Gargill, 1945; Morgans, 1947; Dunlop, 1947; etc.). The average incidence of agranulocytosis in patients so treated appears to be approximately 2 per cent (Moore, 1946; Van Winkle and others, 1946), and 28 per cent of sixty-one cases of this condition reviewed by Morton (1947) died. In view of these and similar findings, it is considered that the results of an investigation of the sternal marrow in cases of thiouracil neutropenia may be of some interest.

A series of six patients who developed neutropenia during the year 1947, while under treatment with methyl (4) or propyl (2) thiouracil, will be considered. The criterion of neutropenia was taken as a peripheral absolute granulocyte count of less than 1,500 per c.mm. of blood (Osgood and others, 1939). A differential count of 500 nucleated marrow cells was made in each case, except in No. 1, in which 1,000 such cells were counted. In none was there evidence of a primary blood dyscrasia which might be associated with an absolute neutropenia. Brief clinical details of these patients are given in the appendix to this report (p. 299).

The Six Cases

Case 1.—The sternal marrow was first examined on April 3, 1947, when only 64 granulocytes per c.mm. were found in the peripheral blood. Stained films showed normal numbers of earlier, with very scanty later, myeloid forms (see Table, Case 1a). There appeared to be a rise in lymphocytes and plasma cells, and a normoblastic reaction (she had hypochromic anaemia; Hb, 70 per cent Hellige—100 per cent=14 g. per 100 ml. blood—on April 3, 1947).

Morphological abnormalities were seen in 55 cells of the myeloid series. Thus some myeloblasts showed, in varying combination, an irregularly shaped nucleus, and scanty, irregularly staining, vacuolated cytoplasm, with irregular outer margin and pseudopodium-like formation (Plate I, Figs. 1 and 2). Promyelocytes with an abnormally large, often irregular nucleus, and cytoplasm showing the features mentioned above, along with coarse granulation (Figs. 3–7), could be seen. The large numbers of cells reported as bare nuclei throughout the series showed, in the main, the general morphology of myeloblastic or premyelocytic nuclei (Fig. 8). Some were degenerated mature granulocytes (Fig. 9).

The cytoplasm of the neutrophil myelocytes showed the abnormal features observed in the premyelocytes, except that the granules were often ill-defined, and seemed to fuse with the cytoplasm (Fig. 10). Irregularity of nuclear outline could be seen in one cell.

The single metamyelocyte encountered also showed irregularly staining cytoplasm with ill-defined granulation, while the neutrophil polymorph exhibited similar granulation, along with an abnormally pale, vacuolated cytoplasm and a pyknotic nucleus.

The sternum was punctured for the second time on April 5, 1947. No granulocytes were then seen in the peripheral blood smears. Examination of the marrow (Table, 1b) showed even more marked myeloid suppression than on April 3, the promyelocyte level being especially affected. In general, the myeloid morphology resembled that seen in the first marrow specimen. Additional features worthy of note amongst the 56 abnormal cells were: one myeloblast showed a partly segmented nucleus, the lobes being joined by a relatively narrow chromatin band (Fig. 11); and the promyelocytes showed the ill-defined granules previously mentioned (Figs. 8, 12, and 13). Some neutrophil myelocytes were abnormally large, and granules were often absent from the cytoplasm, or were of very coarse type (Figs. 14 and 15). A few metamyelocytes showed scanty irregularly staining cytoplasm, with irregular

TABLE

MYELOGRAMS IN THIOURACIL NEUTROPENIA

Cell types	Percentage of cell types								
	From counts of 1,000 cells				From counts of 500 cells				
	Case 1a	Case 1b	Case 1c	Case 1d	Case 2	Case 3	Case 4	Case 5	Case 6
Myeloblasts	2.5	2.4	0.9	0.5	1.6	1.8	2.0	1.8	0.6
Promyelocytes	3.0	1.5	5.0	4.2	7.6	9.4	7.4	2.0	6.8
Neut. myelocytes	0.6	1.6	8.3	19.6	9.8	2.4	5.8	0.8	6.8
Neut. metamyelocytes	0.1	0.6	2.9	21.3	24.0	23.6	18.0	1.4	24.8
Neut. segmented	0.1		0.1	6.5	8.6	12.2	11.6	3.2	19.2
Eos. myelocytes				0.2	1.6	1.0	1.2		0.4
Eos. metamyelocytes									0.2
Eos. segme..ted					0.2	1.2	1.0		
Adult basophils				0.1	·	0.2			
Megakaryocytes	0.1	0.1		0.1			0.2	0.2	0.2
Lymphocytes	23.6	34.2	42.1	23.4	22.8	17.4	6.0	25.4	12.6
Plasmacytes	2.8	2.2	2.0	3.1		0.6	0.8	0.5	1.6
Monocytes	2.5	3.4	0.4	0.7	1.6		0.6	0.6	0.8
Reticulum cells	1.0	0.8	0.1	0.6	0.2	0.2	0.6	0.4	0.2
Cells in mitosis—									
Promyelocytes			0.1	0.1		0.2	0.2		0.2
Neut. myelocytes			0.1	0.1	0.2				
Haemocytoblasts		0.2				0.2	0.4	0.4	0.8
Pronormoblasts	2.0	0.7	0.9·	0.4	0.6	1.0	1.2	1.4	1.8
Basophil normoblasts	9.8	5.5	2.7	0.4	1.4	3.2	8.0	7.2	1.4
Polychromatic normoblasts	15.6	13.0	13.2	5.5	5.0	5.0	11.0	16.6	6.0
Orthochromatic normoblasts	15.4	9.5	5.9	2.4	8.6	2.6	12.0	14.8	5.6
Cells in mitosis—									
B. normoblasts	0.3	0.2	0.4	0.1	0.2		0.2		0.2
P. normoblasts	0.1		5.9	0.2				0.8	
Bare nuclei	20.5	24.1	14.6	10.5	6.0	17.2	11.8	22.5	9.8
Myelo-lymphoid ratio	0.3/1	0.2/1	0.4/1	2.2/1	2.3/1	3/1	8/1	0.36/1	4/1
Myelo-erythroid ratio	0.2/1	0.19/1	0.7/1	6/1	3.4/1	4.4/1	1.5/1	0.23/1	4.7/1

outline, vacuolation, and ill-defined or absent granules (Fig. 16).

A further puncture was performed on April 7, 1947, at a time when granulocytes were beginning to reappear in the peripheral blood smears (64 per c.mm.). The sternal marrow (Table, 1c) showed evidence of myeloid regeneration, and only one abnormal myeloblast with vacuolated cytoplasm could be seen. Many of the promyelocytes showed abnormal features as described above. Some of the neutrophil myelocytes were abnormally large ; the nucleus of some exhibited nucleoli, and the cytoplasm of others, was completely disrupted (Fig. 17). A fresh feature noted in the cytoplasm of metamyelocytes was the coarse granulation ; that of the single polymorph was abnormally pale, with irregular outline and devoid of granules (Fig. 18) (120 abnormal myeloid cells were seen).

The final puncture, performed on April 10, 1947, was made when the peripheral granulocyte count was rising rapidly. The smears showed marked evidence of myeloid regeneration (Table, 1d). All the myeloblasts appeared normal, and only 39 of the remaining myeloid cells showed any of the abnormal morphological features described above.

Case 2.—Sternal puncture was performed on Oct. 30, 1947, peripheral blood smears showing 1,160 granulocytes per c.mm. There appeared to be a " myeloid maturation defect " (Table, 2). The myeloblast morphology was normal, but 25 of the more differentiated myeloid forms showed abnormalities of structure of the types described in Case 1. Additional features worthy of note were 2 metamyelocytes with abnormally large nuclei (Fig. 19), and one eosinophil myelocyte with vacuolated cytoplasm.

Case 3.—On March 26, 1947, 1,200 granulocytes per c.mm. were seen in peripheral blood smears. The marrow (Table, 3) showed a relatively slight apparent myeloid " maturation defect," while 27 myeloid cells exhibited abnormalities of the types noted in the previous cases (Fig. 20).

Case 4.—The peripheral granulocyte level standing at 854 per c.mm. sternal marrow smears obtained on May 20, 1947, showed an apparent " maturation defect " in the myeloid cells (Table, 4). The normoblastic reaction was expected in view of chronic haematuria (on admission she had 50 per cent Hb, Hellige). Worthy of note was the disrupted cyto-

plasm of two neutrophil polymorphs and two eosinophil polymorphs (Fig. 9); otherwise, the 20 abnormal myeloid cells showed no features not already seen in the other cases.

Case 5.—On June 1, 1947, when the sternal marrow was examined, there were 504 granulocytes per c.mm. in the peripheral blood. Again the Table (5) shows a myeloid "maturation defect," and 27 abnormal myeloid cells showed changes described above.

Case 6.—Sternal marrow puncture was performed on Dec. 1, 1947, when 1,250 granulocytes per c.mm. were seen in the peripheral blood. Marrow smears (Table, 6) showed 10 abnormal immature myeloid cells, 1 myeloblast, 2 neutrophil myelocytes, and 7 premyelocytes with neutrophil myelocytic nuclei. Otherwise there was no new feature.

Sternal marrow examinations were carried out on five thyrotoxic patients in whom no neutropenia occurred during thiouracil therapy. In these, neither the morphological abnormalities, nor the marked myeloid depression described in the six neutropenic cases, were observed.

Discussion

The six cases showed varying grades of neutropenia up to complete agranulocytosis in Case 1. The sternal marrow appeared to contain an excess of lymphocytes in Cases 1, 2, and 5, and of plasmacytes in Case 1, these findings being common in cases of neutropenia and agranulocytosis (Darling and others, 1936). The lymphocyte morphology was normal throughout, no cells of the type described by Downey and Stasney (1936) being evident. There appeared to be no significant increase in reticulum cells and haematogones (haemocytoblasts) such as McGavack and others (1944) found in a case of thiouracil neutropenia. The erythropoietic tissue showed no constant variation from normal, as one would expect (Wintrobe, 1946a). In no case was there a megakaryocytic increase, found by Gessler (1946) in two of his series of nine patients. The latter had been treated with thiouracil, and two had developed neutropenia.

The most striking changes were found in the myeloid series; they were constantly depressed (see Table), and displayed morphological alterations as described above. Gessler (1946) and Limarzi and Ricewasser (1946) have reported a few of these, while some of the other abnormalities have been described previously in cases of agranulocytosis not associated with thiouracil (Rotter, 1925; Jaffé, 1933; etc.). There were obvious discrepancies in the maturation of the nucleus and cytoplasm throughout the series (Plate I, Figs. 1, 8, 11, 13, 17, and 19).

The mechanism of the depression of the bone marrow, and of the leucopenia, has by no means

been fully elucidated. Neutropenia appears to depend on the development of hypersensitivity of the white cells to the drug, and other symptoms suggestive of allergy appear from time to time during treatment—for example, skin rashes and drug fevers (Witts, 1936; Plum, 1937; Hadler, 1940; Lesses and Gargill, 1945; Fishberg and Vorzimer, 1945; etc.). Our case of agranulocytosis developed erythema, but none of the cases showed eosinophilia at any stage. Williams and Clute (1944) showed that patients may recover from neutropenia with continued administration of the drug, and desensitization to thiouracil has been noted by several authors (Sprunt, 1944; Rose and McConnell, 1944). However, Williams and others (1944) have shown that human bone marrow is one of the tissues most highly saturated with the drug. They also found that the concentration of thiouracil in the leucocytes was greater than in the erythrocytes. Palmer (1944) supports these findings, and Warren (1945) showed that thiouracil produced a small but significant inhibition of respiration of rabbit bone marrow cells, more especially of the immature myeloid cells. Thus a direct toxic effect of the drug may play a part. It is felt that the morphological abnormalities described above, and affecting even the earliest myeloid cells, and the discrepancies in maturation between the nucleus and cytoplasm strongly support this view. Noteworthy, too, is the chemically close relationship of the thiourea drugs to barbitone or diethylbarbituric acid, which latter occasionally gives rise to agranulocytosis (Watkins, 1933). That the neutropenia does not arise from thyrotoxicosis *per se* has been adequately shown (Lesses and Gargill, 1945), and there is evidence that anoxia does not play an important part (Rosin and Rachmilewitz, 1948).

Whether depressed function of the marrow is due to allergy or to a direct toxic effect and overdosage, or both, actual production of the neutropenia would appear to be explicable by either one or other of the following concepts:

(1) The much favoured "maturation arrest" (Fitz-Hugh and Krumbhaar, 1932; Fitz-Hugh and Comroe, 1933), in which the effect is exerted on the precursors of granulocytes, interfering with their maturation. Thus Rubinstein (1944) performed serial marrow examinations in a case of severe thiouracil neutropenia and came to the conclusion that agranulocytosis was caused by arrest of maturation associated with hypoplasia.

(2) Plum's (1937) evidence favours the view that the bone marrow in agranulocytosis goes through a number of stages. The first is a reduc-

tion of granulocyte precursors, soon followed by a loss of mature and then immature granulocytes. Recovery is heralded by the reappearance of granulocyte precursors, the picture then being one of so-called " maturation arrest." Later, the mature granulocytes return. (It is of interest to note that Jaffé, 1933, described three cases of idiopathic agranulocytosis, and one following anti-luetic treatment. The femoral marrow at autopsy was hyperplastic, the granulopoietic tissue taking an active part. He thought, therefore, that in some instances the agranulocytic catastrophe was preceded by proliferation of young myelocytes.) The work of Braun (1944), of Dameshek (1944), and of Sikkema and others (1946) supports this second view.

Our cases would also seem to favour the Plum concept, both from the evidence of a direct toxic effect already mentioned and from that afforded by the differential marrow counts in the Table. There is evidence of proliferation of earlier myelocytes in most cases, and in Case 1 this preceded the development of the more mature granulocytes. Also in favour are the large number of " bare nuclei " showing the general morphology of myeloblastic or premyelocytic nuclei. We must remember, however, that destruction of some of the granulocytes may occur in the circulating blood (Lawrence, 1941).

Fisher (1947) pointed out that neutrophil leucopenia occurred in his series of cryptogenic acquired haemolytic anaemias only in those cases with liver dysfunction. In retrospect, he noted that neutropenia was the only early and constant herald of liver dysfunction. It is interesting to speculate about the hepatic role in thiouracil neutropenia. The liver is affected in hyperthyroidism. There is often passive congestion, and degenerative changes are common. Fatty change, acute necrosis, both focal and central, and subacute toxic atrophy with the development of cirrhosis occur frequently. Liver function tests often show marked impairment (Weller, 1930 ; Beaver and Pemberton, 1933 ; Boyd, 1943). In addition, thiouracil therapy may be associated with jaundice and hepatic enlargement (Kahn and Stock, 1944 ; Paschkis, 1944 ; Sloan and Shorr, 1944 ; Lesses and Gargill, 1945 ; Linnell and others, 1946 ; Livingston and Livingston, 1947). Case 5 had hepatomegaly, but all the generally accepted liver function tests were normal, except the galactose tolerance test, which gave a value of 4.5 g. excreted in five hours after 40 g. had been taken by mouth. Case 3 showed a similar result, and a serum alkaline phosphatase of 19 K-A units. Otherwise, the usual liver function tests were normal throughout our series. This

latter does not support the view that liver function has much to do with thiouracil neutropenia.

Peripheral blood films in the six cases showed varying grades of degeneration of some granulocytes. Some had a pyknotic nucleus, or a pale nucleus with no cytoplasm but surrounded by granules. Vacuolation of the cytoplasm, abnormal bluish staining, and coarse and fine poorly staining granules were seen. In no case was a leucocytosis detected on recovery (cf. Reznikoff, 1938 ; Lesses and Gargill, 1945 ; Kneedler, 1946 ; etc.).

Bacteriological cultures of the marrow under aerobic (blood agar, glucose broth) and anaerobic (blood agar and Robertson's meat medium) conditions were carried out in Cases 1, 3, 4, and 5. After fourteen days' incubation they remained sterile. In each case the marrow sample was taken before the commencement of penicillin therapy. These findings support the view that the neutropenia is not primarily due to an infection (Wintrobe, 1946b).

Our cases (see appendix) confirm the fact that no general rules will guarantee freedom from serious haematological reactions. Neutropenia was seen at very different dosage levels, and after short and prolonged periods of administration. Clinical symptoms are not reliable as an indication of the blood picture ; thus Case 5 has continued over the past year free from symptoms, with a granulocyte count remaining at about the 1,000 per c.mm. level.

Summary

The sternal marrow pictures of one patient who developed agranulocytosis and of five who developed neutropenia while under treatment with thiouracil derivatives are described, with particular reference to numerical and morphological changes in the myeloid series, which was constantly depressed.

The mechanism of this depression is discussed, and evidence in favour of a direct toxic effect of the drug is given. The morphological abnormalities described, affecting even the earliest myeloid cells, and the discrepancies in maturation between the nucleus and cytoplasm strongly support this view.

The mode of production of thiouracil neutropenias is better explained by the Plum (1937) concept than by that of " maturation arrest."

The liver function tests performed on these cases do not uphold the view that liver function has much connexion with thiouracil neutropenias.

Bacteriological cultures of the sternal marrow were sterile in four cases examined by suitable methods. This negatives infection as a primary cause of the neutropenias.

It was confirmed that no general rules will guarantee freedom from serious haematological reactions, and clinical symptoms are not reliable as an indication of the blood picture.

I wish to thank Prof. H. N. Green and Dr. L. C. D. Hermitte for their kind encouragement and advice; Prof. E. J. Wayne and Dr. H. P. Brody for their willing assistance, and for access to their cases; and Drs. W. D. Wallace, R. H. Canter, and J. F. Goodwin, Mrs. G. R. Maclachlan, and the technical staff for help with various aspects of the work.

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Appendix

Case 1.—A married woman aged 62 years was admitted to hospital with typical thyrotoxicosis and congestive heart failure. Methyl thiouracil, 0.2 g. three times a day, was begun on March 12, 1947. The granulocytes fell from 2,120 on March 5 to 1,122 per c.mm. on March 12, after which a satisfactory level was maintained until March 31, when a value of 1,188 per c.mm. was found. On March 24 thiouracil therapy had been stopped for twenty-four hours in view of a transient mild sore throat. The granulocytes fell to 64 per c.mm. (total leucocytes 3,200 per c.mm.) by April 3; the drug was again stopped, and the first sternal puncture performed. Treatment with penicillin, intravenous pyridoxine, and intramuscular pentnucleotide was begun, her only fresh symptom being a slight sore throat.

A few hours after the first sternal puncture on April 3 she complained of malaise, headache, pains in her back and limbs, and a sore throat. At 6 p.m. she had a rigor, her temperature rising to 101° F. She appeared acutely ill, with flushed facies and coated tongue. Her fauces were injected, but no exudate was evident. Slightly enlarged, tender, tonsillar lymph nodes were palpated. There was no splenic or hepatic enlargement, and no bone tenderness, icterus, or skin rash.

The following day the temperature remained intermittent, and the peripheral granulocyte level stood at 60 per c.mm., with a total leucocyte count of 3,000 per c.mm. At 10.40 p.m. she experienced a rigor, this being thrice repeated on April 5, on which day she developed generalized erythema, and had a leucocyte count of 2,800 per c.mm., no neutrophil polymorphs being evident in peripheral or marrow films.

The complete neutropenia was maintained on April 6, and she had a rigor at 10 p.m., which was repeated the next day at 3 p.m., when her peripheral blood contained 64 granulocytes per c.mm. Another sternal marrow examination was made on April 7.

Marked clinical improvement took place on April 8, this being reflected in her peripheral blood, which showed 704 granulocytes (64 neutrophil myelocytes, 96 metamyelocytes, and 544 polymorphs), out of a total of 3,200 leucocytes per c.mm. The following day no neutrophil myelocytes were seen, and the granulocyte level rose to 924 per c.mm. The final marrow smears were examined on April 10, 1947.

Thereafter, the patient's leucocyte counts were satisfactory, and methyl thiouracil 0.2 g. twice a day was recommended on April 29. Agranulocytosis which, apart from a moderate rise of temperature, was symptomless developed suddenly on May 19. The drug was stopped and she recovered on suitable treatment.

Eight months later, in view of increasing toxicity, and the previous reactions to methyl thiouracil, thyroidectomy was performed after suitable preoperative treatment. The patient died a few hours later in a thyroid crisis.

Case 2.—In 1941 a married woman aged 45 years had a subtotal thyroidectomy for typical thyrotoxicosis. In view of recurrent toxic symptoms, methyl thiouracil 50 mg. per day was begun on Aug. 8, 1946. The dose was reduced to 25 mg. daily on Oct. 31, being raised again to 50 mg. on March 27, 1947. Her granulocyte count was satisfactory until Oct. 24, 1947, when a leucopenia (2,600 per c.mm.) and neutropenia (936 per c.mm.) were found. The granulocyte level varied between 936 and 1,160 per c.mm. over the next few symptomless days, thiouracil therapy being stopped and penicillin and pyridoxine exhibited on Oct. 26, 1947. Sternal puncture was performed on Oct. 30, 1947.

Case 3.—A married woman aged 38 years was admitted to hospital with auricular fibrillation and congestive heart failure due to primary thyrotoxicosis. Propyl thiouracil 50 mg. three times a day was given for fourteen days, 25 mg. three times a day for the next five days, and then 25 mg. twice daily for seventeen days, after which (March 20, 1947) 25 mg. daily sufficed. Her total leucocyte count varied between 3,000 and 3,600 per c.mm. from March 17 until March 26, when her sternal marrow was examined. Previous counts had been normal, and she never had symptoms attributable to neutropenia.

Case 4.—A married woman aged 43 years came into hospital on April 28, 1947, suffering from thyrotoxicosis and left pyonephrosis. Her leucocyte count was 2,400 per c.mm. with 1,584 granulocytes per c.mm. Propyl thiouracil, 50 mg. three times a day, was given, and she developed more marked leucopenia, which was symptomless and persisted for four days before her sternal marrow was examined on May 20. On this date the peripheral blood showed 1,400 leucocytes and 854 granulocytes per c.mm.

Case 5.—A man aged 53 years, a case of primary thyrotoxicosis, was given methyl thiouracil 200 mg. per day for one month, and then 100 mg. daily for three and a half months, followed by 25 mg. daily for one year. He then developed leucopenia (3,800 per c.mm.) due to neutropenia (418 granulocytes per c.mm.) associated with palpable enlargement of liver and spleen on May 31, 1947. He had no pyrexia, sore throat, skin rash, adenitis, icterus, bone tenderness, or anaemia, the neutropenia being symptomless.

Case 6.—A married woman aged 49 years had had a thyroidectomy four years previously for toxic goitre. She relapsed, and there was a concomitant B. coli pyelonephritis. On admission on Nov. 28, 1947, she had hypochromic normocytic anaemia (Hb, 67 per cent Hellige), and leucopenia (2,000 per c.mm.) and neutropenia (1,220 granulocytes per c.mm.). The following day methyl thiouracil, 200 mg. twice a day, was begun, and her marrow was examined on Dec. 1, there having been no symptoms attributable to the neutropenia.

LEGENDS FOR PLATE 1

(All the photomicrographs are Leishman-stained, and the magnification is × 1,000)

FIG. 1.—Myeloblast showing an irregularly shaped nucleus and irregularly staining cytoplasm with interruptions in the continuity of its outer margin.

FIG. 2.—Degenerating myeloblast showing scanty, irregularly staining cytoplasm, with irregular outer margin, and pseudopodium-like formation.

FIG. 3.—Large promyelocyte with a few coarse cytoplasmic granules.

FIG. 4.—Large promyelocyte showing a cytoplasmic vacuole, and irregularity of cytoplasmic staining and outline.

FIG. 5.—Promyelocyte showing irregularly staining cytoplasm, which contains coarse granules and a vacuole.

FIG. 6.—Promyelocyte showing irregularly staining cytoplasm and coarse granules.

FIG. 7.—Promyelocyte showing irregularly staining cytoplasm with irregular outer margin and pseudopodium-like formation. Some coarse cytoplasmic granules are seen.

FIG. 8.—Nucleus only and promyelocyte, showing morphological similarities. The promyelocyte also shows an irregularly shaped nucleus, and ill-defined granules, which seem to fuse with the cytoplasm.

FIG. 9.—Degenerate polymorphonuclear cell showing a stage in development of "nucleus only."

FIG. 10.—N. myelocyte showing ill-defined granules apparently fusing with the cytoplasm.

FIG. 11.—Myeloblast showing partially segmented nucleus.

FIG. 12.—Promyelocyte with ill-defined granules apparently fusing with the cytoplasm.

FIG. 13.—Promyelocyte with irregularly shaped nucleus and granules as in Fig. 12.

FIG. 14.—N. myelocyte showing vacuolated cytoplasm with absent granules.

FIG. 15.—Large N. myelocyte with coarse cytoplasmic granules.

FIG. 16.—Metamyelocyte with scanty irregularly staining vacuolated cytoplasm and ill-defined granules.

FIG. 17.—N. myelocyte. The nucleus contains nucleoli. The irregularly staining cytoplasm has an uneven outer margin and contains coarse granules.

FIG. 18.—N. polymorph with abnormally pale cytoplasm, which has an irregular outline and is devoid of granules.

FIG. 19.—Metamyelocyte with abnormally large nucleus and ill-defined cytoplasmic granules.

FIG. 20.—Eosinophil myelocyte with disrupted cytoplasm.

PLATE I
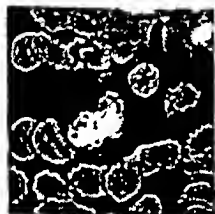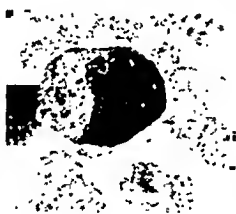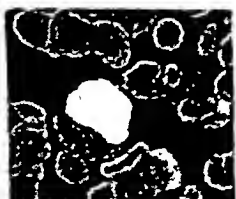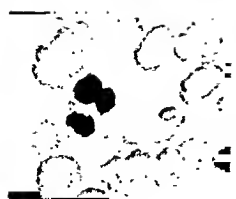
CONGENITAL DEFECTS FOLLOWING RUBELLA

REPORTS OF TWO CASES, ONE OF WHICH SHOWS A HITHERTO UNDESCRIBED LESION

BY

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The association of maternal rubella in the third month of pregnancy with ocular defects in the infant was first noticed in New South Wales by Gregg (1941). Subsequently (1946) the matter was investigated by Swan and his collaborators in South Australia. Further investigations have been made in the United States and in this country. By now there have also been reports of maternal rubella preceding the following defects in the infant: congenital abnormalities of the heart, abnormal size of the head, deafness, feeding difficulties, subnormal weight.

Apart from records by Swan (1944) very few post-mortem observations have been made. His " Final Report " (1946) mentions " heart disease " in a number of the cases, but there are no further post-mortem records. We have been unable to trace any such records in this country. The already voluminous literature on the subject deals in the main with case reports, probabilities, and theories of mechanism.

The purpose of the present paper is to record two further instances in which post-mortem examinations were made and to discuss relevant findings.

Case Reports

Case 1.—An infant was born to a primipara of 26 years. It was known that the mother had had rubella when ten weeks pregnant. The delivery was normal, but the child lived for only fifteen minutes.

Post-mortem findings. — Full-term female child weighing 6 lb. There were no external abnormalities. The body was radiographed, but no bony abnormalities found. The eyes were bisected and found normal. Cranium and brain were also normal.

The thorax and abdomen were anatomically grossly abnormal (Plate II). The small bowel and the left lobe of the liver (1) were seen to be in the thoracic cavity. The heart (2) and lungs were deviated to the right, and both lungs were much compressed. A thin edge of both lobes of the left lung ran diagonally across the thorax (3) from the apex of the cavity to the lower right-hand corner, where it met the displaced heart and pericardium. A large thymus gland (4) covered the upper portion of the pericardium and filled the whole of the superior mediastinum.

The right muscular portion of the diaphragm was well formed (5, 16). The posterior muscular portion of the left half of the diaphragm was present (17), but there was no anterior two-thirds of the left half of the diaphragm (6). Through the gap thus made had herniated the left lobe of the liver, the stomach and nearly all the small intestines, the spleen, the pancreas, and in an extraordinary fashion the bulk of the colon.

The caecum and appendix were to the left of the mid-line in the epigastric region (7). The ascending colon (8) traversed the left side of the liver and stomach to reach the top of the left thoracic cavity. It descended (9) antero-superiorly to the spleen (21), which itself lay on the left diaphragm, to come into more normal lateral relationship with the left kidney (13).

The pelvic colon (10) was unnaturally mobile and ran from the left iliac fossa upwards and then across in a wide sweep to the right iliac fossa, where it entered the pelvis minor on the right side. In its sweep it embraced the left ovary and fallopian tube which lay on its mesentery to the right side of the mid-line. The uterus (11) was rotated through 30 degrees, so as to face to the left.

The heart (2) was of normal size and shape, although it lay in the right thoracic cavity.

There was a large patent inter-auricular septum, but no interventricular communication. The pulmonary artery was of average size, both in length and diameter. The ductus arteriosus was more prominent and of wider lumen than the aorta. It was 2 cm. in length from the origin of the left pulmonary artery to its junction with the aorta beyond the left subclavian artery. It formed a complete right angle with the descending aorta, and ran almost parallel to the ascending aorta in the manner shown (see diagram).

In its descending and abdominal portions the aorta appeared to be normal in length, lumen, and anatomi-
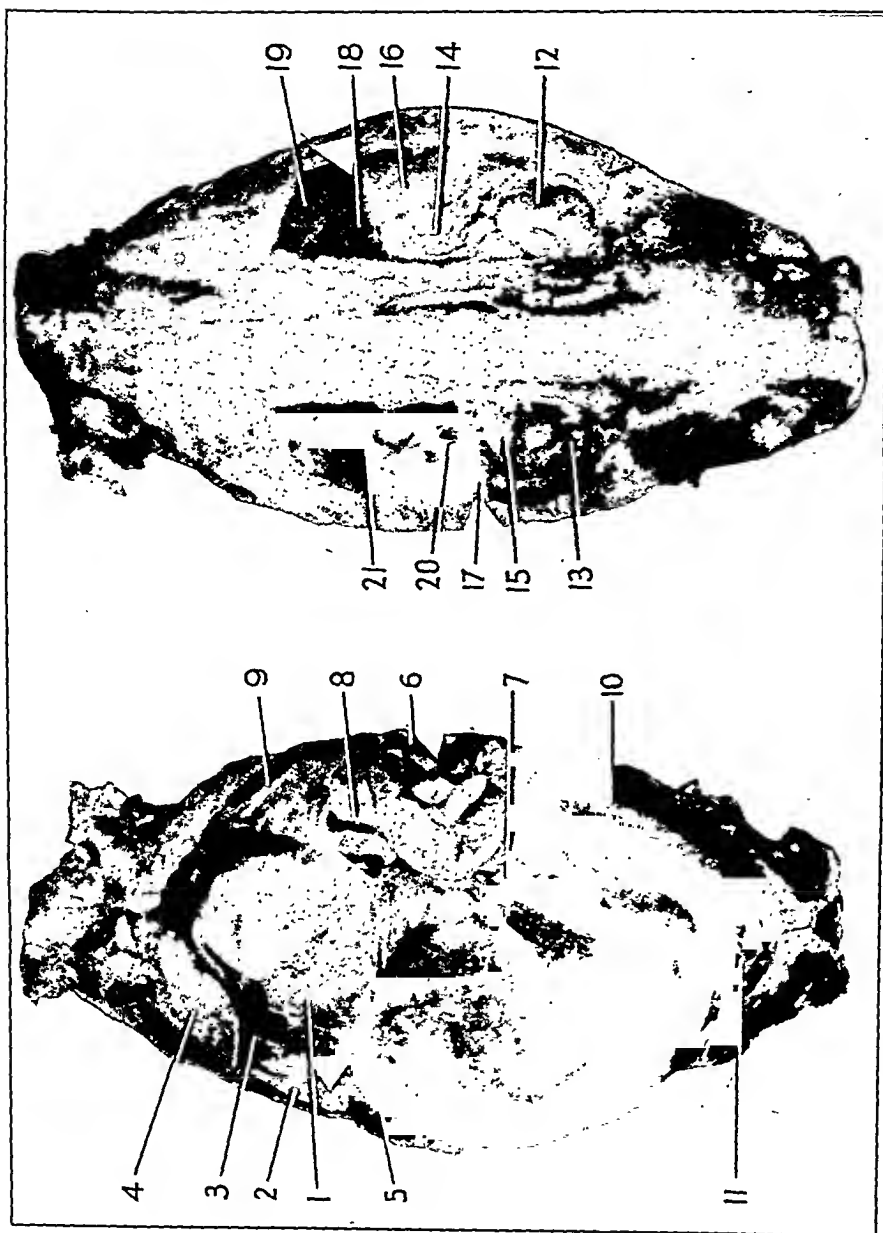
PLATE II.—(*Left*). Case I viewed from the front. The area between the white triangular pointers indicates the gap in the diaphragm. Key = (1) left lobe of liver ; (2) heart ; (3) anterior edge of left lung ; (4) thymus ; (5) right half of diaphragm ; (6) rudimentary left diaphragm ; (7) caecum and appendix ; (8) ascending colon ; (9) descending colon ; (10) pelvic colon ; (11) uterus.

(*Right*). Posterior view. Key = (12) right kidney ; (13) left kidney ; (14) right adrenal ; (15) left adrenal ; (16) right diaphragm ; (17) left diaphragm ; (18) lower lobe right lung ; (19) middle lobe right lung ; (20) descending colon ; (21) spleen.

cal position. Although the traehea was anatomieally displaced to the right, it was normal in size and shape.

The oesophagus maintained normal relationship with the aorta until its emergenee at the diaphragm. There was no diaphragm to the left and anteriorly, although there was a thin museular slip to the left posteriorly, and a fully formed diaphragm to the right.

The stomaeh lay along the left border of the dorsal vertebrae and it was of normal size and shape. No abnormality of pylorus or duodenum was observed.
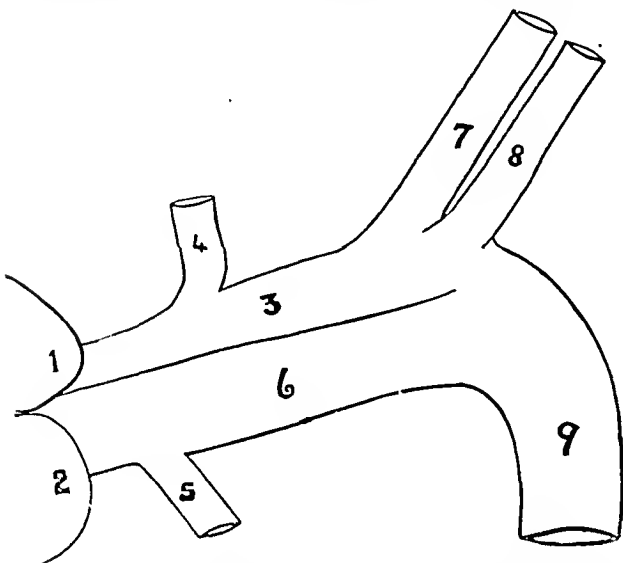
Relations between the aorta, pulmonary artery, and ductus arteriosus in Case 1. Key = (1) right auricle; (2) left auricle; (3) ascending aorta; (4) innominate artery; (5) left pulmonary artery; (6) ductus arteriosus; (7) right carotid artery; (8) subclavian artery; (9) descending aorta.

The kidneys (12 and 13) were normal anatomically and histologically. So were the ureters and bladder. The adrenal glands (14 and 15) were normal in size, shape, and position. The panereas was displaeed upwards into the left thoraeic cavity. The spleen (21) lay on the thin slip of left diaphragm within the chest wall.

Case 2.—A male baby of three days was admitted with his mother to hospital beeause of marked oedema of the lower half of the body, jaundiee, vomiting, and loose stools. This infant, the seeond ehild, was born at home. Delivery had been normal, and the weight at birth was 6 lb. 9 oz.

Two events had interrupted the otherwise normal eourse of pregnaney. Towards the end of the third month of the pregnaney the mother had contracted rubella. The infeetion was mild and did not appreei-ably affect her general eondition. At four months she was threatened by abortion. After a few days' rest in bed the danger passed, and the further eourse of the pregnaney remained uneventful.

The first ehild, aged 15 months, was well. The parental history did not reveal anything relevant.

Clinical findings.—The infant was normal, well developed, slightly jaundieed, with extensive oedema of feet, legs, scrotum, and genital area, saeral region, and lower abdominal wall. Heart, lungs, abdomen, urine, and blood were all normal. Bilateral cataracts were notieed a few days after admission. Apart from a large anterior fontanelle the skull was normal.

In the course of the following week oedema sub-sided and the jaundiee cleared completely; the infant took the breast well and started to gain weight. On the tenth day after admission mother and baby left hospital.

Fourteen days later the infant, now artifieially fed, developed fulminant bronehopneumonia and paren-teral gastro-enteritis, and was readmitted in a mori-bund eondition. He died within thirty-six hours.

Post-mortem findings.—The body appeared norm-ally formed but was small. There was slight dehydra-tion. There was bilateral congenital eataraet. Out of deference to the parents the eyes were not removed. The eause of death was bilateral pneumonia, more advaneed in the right lung. The heart was typieally "sabot" shaped. There was dilatation of the right chambers. The ductus arteriosus was widely patent, its lumen not mueh less than that of the pulmonary artery, although the wall was thinner. Its endothelium was smooth, unwrinkled, and directly continuous with that of the larger arteries. Microscopic examination did not show any evidenee of obliterative endarteritis in the patent duetus arteriosus. No abnormality was found in the glomeruli of the kidneys.

Discussion

The association of rubella in the first three months of pregnancy with congenital defects in the foetus has been noted by Clayton-Jones (1947), Hughes (1945), and Hope-Simpson (1944) amongst others in this country. Most investigations have been made by tracing histories from children show-ing congenital defects to a maternal infection (Swann, 1944; Clayton-Jones, 1947; Conte and others, 1945; Erickson, 1944).

A different line of investigation was followed by Fox and Bortin (1946). They reviewed the noti-fications of rubella in a certain community over a period of three years. The total number of cases in all classes of people was more than 22,000. Of 152 married women notified, eleven were pregnant. Nine of these were in the first four months of their term. In these cases there was one twin birth, one blue baby with hydrocephalus which receded spon-taneously, and one stillborn hydrocephalic. The remaining children were normal.

Parsons (1946a) states that causal relationship between maternal rubella and congenital defeets has not been definitely proved, and in a further paper (1946b) he discusses some of the difficulties of the theory. Swan (1944) describes patency of the ductus arteriosus in his series of babies coming

to necropsy. Gregg is quoted (Parsons, 1946b) as finding forty-four cases of congenital heart disease out of seventy-eight affected infants.

Other abnormalities described have been mental retardation, microcephaly, mongolism, hypospadias, talipes equino-varus, and synostosis of radius and ulna or tibia and fibula.

Congenital diaphragmatic hernia has not hitherto been described in the literature as one of the developmental defects occurring in association with maternal rubella.

The diaphragmatic hernia in Case 1 differs from the common form, in which all diaphragmatic segments are present but a patency exists between the pleural and peritoneal cavities. This pleuroperitoneal hiatus, or foramen of Bachdalek, may vary in size, but it is surrounded by diaphragmatic elements. Abassy has quoted Dickson as saying that diaphragmatic hernia is seen in one case out of each hundred routine gastric radiographs.

In this case there was a total absence of the anterior two-thirds of the left half of the diaphragm. There was, however, a small muscular slip to represent the posterior third of the diaphragm.

Developmentally the diaphragm arises in four main parts: a ventral, a dorsal, and a right and a left lateral. The ventral part is formed from a septum transversum which is gradually differentiated into a caudal, an intermediate, and a cephalic part. It is from the intermediate portion that the ventral part of the diaphragm is formed. The dorsal portion of the diaphragm arises from the mesoderm of the dorsal mesentery of the foregut. The two lateral portions grow towards the median plane until they fuse with the dorsal portion.

The present appearance of the diaphragm would suggest that there had been a failure of growth of the left lateral portion and of the left half of the ventral portion. It resulted in a very rudimentary left half of the diaphragm, and this gross diaphragmatic hernia.

The time of formation of the diaphragm from its various elements is from the eighth week onwards, after the pericardial cavity has already been shaped. The inter-atrial and interventricular septa begin to develop between the fourth and sixth weeks. The former is the result of the coalescence of the primary and secondary septa. Their fusion is partial and leaves the foramen ovale. The valve of the foramen is formed in the fourth month. The interventricular septum arises in the lower part of the primitive ventricle, and grows upwards to meet the endocardial cushions and the septum developing in the bulbus arteriosus. The lower

part is complete about the eighth week, and the septum of the bulbus arteriosus a few days earlier.

In Case 1 the mother was stated to have suffered from rubella when ten weeks pregnant. The time of formation of the diaphragm is about the eighth week. The possibility that the maternal disease and the time of formation of the affected part coincided is not to be lightly dismissed.

In Case 2 the mother suffered from rubella at about the third month. The ductus arteriosus forms about the sixth week ; the valve of the intra-atrial septum about the sixteenth week ; the inter-atrial septum about the eighth week. Thus the infection would have had ample opportunity to interfere with the development of the cardiac septa.

Parsons (1946b) has canvassed the possibility of strain differences, as well as emphasizing the difficulties of diagnosis. This is a point which most authors in this country stress. Some support for the theory is given by Conte and others (1945), who found that the incidence of rubella in the appropriate months of pregnancy in mothers of children with congenital abnormalities was ten times the expected rate for women of the child-bearing age of the population at large.

Swan and others (1946) do not deal only with rubella as an antecedent of congenital defect. They list cases in which influenza, scarlet fever, herpes zoster, varicella, and mumps, together with one of " pustular rash," were antecedents.

Summary

Post-mortem records of two infants with congenital malformations following maternal rubella are described. Case 1 presented an unusual form of congenital diaphragmatic hernia and a widely patent ductus arteriosus. Case 2 had bilateral congenital cataract and a wide patency of the ductus arteriosus.

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STUDIES ON TETANUS PROPHYLAXIS

BY

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Before the second world war several investigators had demonstrated the possibility of actively immunizing man against tetanus (Ramon and Zoeller, 1927 ; Jones and Moss, 1937 ; Marvell and Parish, 1940 ; Wolters and Dehmel, 1940). During the war this method was tested on a large scale, for in the allied armies prophylaxis against tetanus was based almost entirely on active immunization. Reports so far clearly indicate its value and its superiority to serum prophylaxis (Boyd and MacLennan, 1942 ; Long, 1946 ; Ramon, 1946).

Comparison between Active and Passive Immunity

The amount of antitoxin per ml. of serum required for protection against tetanus after active immunization of man can be estimated in at least two different ways: (1) by determining the level of antitoxin capable of protecting actively immunized animals against artificial infection ; (2) by consideration of the amount of antitoxin known to be generally effective in passive prophylaxis against tetanus in man.

The first method takes no account of the difference in susceptibility to tetanus toxin between man and animal nor to the difficulties in estimating the amount and rate of toxin production in the artificial infection or its relation to that occurring in naturally sustained injuries. Since in the pathogenesis of tetanus the intoxication predominates and the infection plays a minor part, the total amount of antitoxin available as well as its concentration must be of great importance, and no direct conclusions should be drawn from experiments on small laboratory animals.

Accordingly we adopted the second method, which depends on the established fact that 3,000 I.U. of antitoxin gives effective protection against tetanus for eight to twelve days after injection.

Titrations of tetanus antitoxin in serum after passive immunization have been reported by Sneath (1934) and Gold (1941). Three days after injection of 3,000 I.U. the serum antitoxin level lay between 0.2 and 0.5 I.U. per ml. ; a week after injection it lay between 0.02 and 0.2 I.U. per ml.

In order to estimate the passive immunity the antitoxin titre in the serum of a number of out-patients was determined at varying intervals after the intramuscular injection of 3,000 I.U. of unrefined tetanus antitoxin, which was given as for routine prophylaxis after accidental injuries. (This work, begun in 1943, is referred to in a paper by Ericsson and others, 1944.) The titrations were carried out by a micro-method, a modification of the standard method used for routine purposes in the manufacture of the commercial products of this laboratory.

Three times the quantity of tetanus toxin, which in titration against international standard serum has been found to correspond to 0.005 I.U., is mixed with 1.5 ml. of undiluted serum or serum dilution. One third of the mixture is injected into each of two 16-g. mice. If both survive, the serum sample or dilution is considered as containing more than 0.005 I.U. per 0.5 ml.

The method thus allows the determination of a minimum concentration of 0.01 I.U. tetanus antitoxin per ml. The results for 56 bleedings are given in the Figure. On the Y axis the absolute
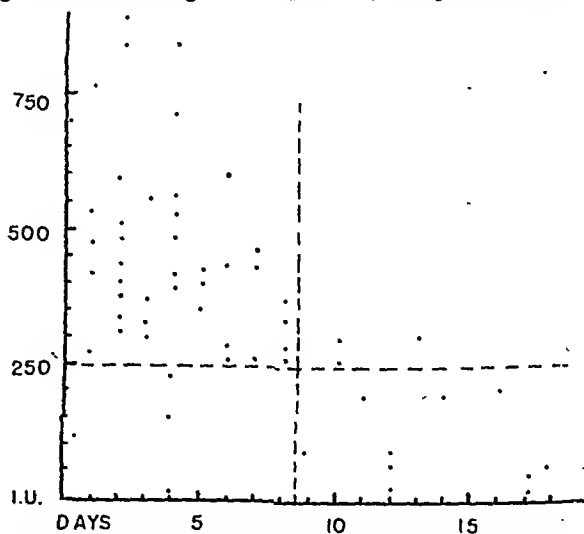
FIGURE.—Showing the total amount of antitoxin available in serum after the injection of 3,000 I.U.

amount of antitoxin available in the patient's circulation is plotted, on the assumption that the patient has 40 ml. of serum per kg. of body-weight.

The values obtained vary considerably from one patient to another. During the first eight days 40 out of 43 values lie above 250 I.U.; during the following days only 3 out of 13 exceed that value. Having assumed that passive immunity is effective for eight days, we concluded that satisfactory protection against tetanus requires 250 I.U. of circulating antitoxin, corresponding to 0.1 I.U. per ml. serum in a 65-kg. man. Such a titre should offer reasonable protection against tetanus in man, and if the same titre is achieved by active immunization a broad safety margin is gained through the inherent superiority of active immunity.

There was no opportunity of titrating serum antitoxin in patients given a second injection of antitoxin. If the second injection is from an animal species different from the first, the rate of loss of antitoxin should not differ from that in the first injection. The opportunities of using serum from different animal sources are few, and consequently for second passive immunization serum from the same species often has to be used. Though the risks of disagreeable and even dangerous reactions after such a procedure have long been recognized, less attention has been paid to the reduced efficacy of the second dose. According to general rules of immunology, one would expect the rate of loss of antitoxin after the second dose of heterologous serum from the same species to be greater than that after the first dose, since a greater capacity for eliminating heterologous serum must be present at the second injection. The practical importance of this has been pointed out by Ramon and his co-workers (1939), but there are very few direct observations bearing on this question. Since the estimation of the efficacy of passive immunization is based on primary injections, the comparison between active and passive prophylaxis should be based on primary injections of antitoxic serum.

Active Immunization

The adsorbed toxoid used in these investigations is that produced regularly at the State Bacteriological Laboratory. It is prepared according to a method primarily elaborated for the production of adsorbed diphtheria toxoid. This method has already been described (Ericsson, 1946).

The method involves precipitation of toxoid with trichloracetic acid and resolution of the precipitate in Na_2HPO_4. The addition of $AlCl_3$ to this solution gives a precipitate of $AlPO_4$ to which the active substance is adsorbed. No washing of this precipitate is

done. The difficulty of obtaining accurate flocculation of the purified preparation prevents an exact determination of the amount of active substance in the final product. However, in view of the titre of 10 to 15 Lf of the raw material, the content of the final product may be estimated at 30 Lf/ml. Guineapigs weighing 250 to 300 g., to which 1 ml. of toxoid is given (the dose designed for human use), will give a titre of more than 1 I.U./ml. of serum in more than half the animals after six weeks.

Ninety-seven healthy men, 20 years of age, who had not received an anti-tetanus inoculation were used in the following experiment. A preliminary test of every fourth person failed to show the presence of naturally occurring tetanus antitoxin in any serum. A single injection of tetanus toxoid was given to each man, followed 6 months later by a booster dose. Table I shows the titres of antitoxin achieved.

TABLE I

SHOWING THE RESULTS ACHIEVED BY ACTIVE IMMUNIZATION

Immunization	Number of patients with the following amount of antitoxin in serum (I.U./ml.)				
	<0.01	0.01–0.1	0.1–1.0	>1.0	Not tested
No immunization	25				72
1 month after single dose	16	64	17		
6 months after single dose	1	23	43		30
4 days after booster dose	1	8	68		20
8 days after booster dose	1			76	20

Bleedings one month after the injection show that it is impossible to obtain, within this space of time, an immunity comparable to that produced by passive immunization. The bleedings performed five months later disclosed a marked increase in the immunity, proving that the antigenic stimulus by adsorbed toxoid had continued for more than a month. These experiments on man confirm the findings of Faragó (1935), who, when immunizing guinea-pigs, showed that the depot of adsorbed toxoid retained its antigenic properties for thirty-eight days.

The immunity brought about by active immunization with toxoid is inferior even after one month to the best result achieved by serum prophylaxis. Therefore primary active immunization is of no value in the case of an injury unless healing is likely to be so protracted that there is a risk of tetanus even after one month.

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The production of antitoxin following the booster dose was of quite a different magnitude and appeared much more rapidly than the antitoxin production after the primary injection (Glenny and Südmersen, 1921). Within four days, that is, within the probable incubation period of tetanus, the booster dose gives an immunity that equals that of the usual passive prophylaxis and, during the following days, surpasses the level of passive immunity. In a person who has already been subjected to basal active immunization a booster dose of toxoid may thus be substituted for the passive prophylaxis.

The results obtained from these studies indicate that the adsorption of the active substance on the precipitate is not so complete as to impair the effectiveness of the toxoid administered as a booster dose. These experiences seem to contradict Miller and Humber's (1943) results, according to which fluid toxoid and not an alum-precipitated one should be used for the booster dose. However, the difference may be explained by the different method employed for the production of the Swedish toxoid. The alum precipitation method usually includes a thorough washing of the precipitate, and it may be assumed that all the activity present is fixed to the precipitate. According to our method the precipitate of $AlPO_4$, the formation of which seems to be the main feature of both the methods, is obtained through the addition of $AlCl_3$ to the solution of $NaPO_4$. Since the purification takes place at an earlier stage in the procedure, and no irritating products are formed by the precipitation, no washing is necessary. Thus the method gives a certain amount of active substance in the supernatant fluid. The difficulty of obtaining accurate flocculations of the toxoid prevents, as already stated, exact determinations. In diphtheria toxoid prepared according to the same method, about one-fourth of the active substance remains in solution, and approximately the same proportion may be assumed to remain in tetanus toxoid. The fact that a certain amount of the active substance of our adsorbed toxoid remains in the solution should be regarded as an advantage, rendering it suitable for the booster dose as well as for the primary injection.

Combined Passive and Active Immunization

Since active immunization is of no immediate value to an injured individual not previously subjected to basal immunization, and as we had to take into account the possible use of serum prophylaxis in the future, it was essential to examine the possibility of attaining a basal active immunity simultaneously with the application of serum prophylaxis.

Investigations performed by Otten and Hennemann (1939) on guinea-pigs, and by Cooke and Jones (1943) on man, have established that passive immunization impairs the development of active immunity.

Preliminary experiments on guinea-pigs were made with our toxoid. Nineteen animals were injected simultaneously but into different sites with 1 ml. of toxoid and 3,000 I.U. of antitoxin (horse). Bleedings were taken on the fourteenth, twenty-eighth, and forty-second days. A control group of six animals was subjected to injections with toxoid only, and the animals were bled on the forty-second day. The results are shown in Table II.

TABLE II
SHOWING THE RESULTS ACHIEVED BY COMBINED PASSIVE AND ACTIVE IMMUNIZATION OF GUINEA-PIGS*

Test animals	Number of animals with the following amount of antitoxin in serum (I.U./ml.)			
	<0.01	0.01–0.1	0.1–1.0	>1.0
Test animals:				
14th day		3	7	9
28th ,,		19		
42nd ,,	13	6		
Control animals:				
42nd day			1	5

* The test animals were given 1.0 ml. of toxoid and 3,000 I.U. of antitoxin simultaneously. The control animals were given 1.0 ml. of toxoid only.

They clearly prove the inhibition of active immunization by the heterologous passive immunity, brought on by the simultaneous injection of antitoxic horse serum.

Clinical trials have also been carried out. Since the beginning of 1944 all the patients at the out-patients department of the surgical clinic of the Caroline Hospital, Stockholm, suffering from injuries suspected of being infected with tetanus, were given 1 ml. of toxoid subcutaneously and 3,000 I.U. of horse serum intramuscularly. Two to three years later, seventy of these patients were examined to determine their antitoxin serum titres and the effect of a booster dose of toxoid. At the first examination, bleedings were made for titration of antitoxin in serum, and at the same time a booster dose of 1.0 ml. of toxoid was administered. The effect of the booster dose was determined by further serum titrations four to nine days later. Only thirty-six patients reported for the second control examination. The results are indicated in Table III.

Out of seventy persons tested, thirty-two showed direct evidence of residual immunity as demonstrated by a measurable titre of antitoxin in serum

TABLE III

SHOWING THE REMAINING TITREABLE IMMUNITY IN PATIENTS TWO TO THREE YEARS AFTER COMBINED PASSIVE AND ACTIVE IMMUNIZATION AND THE EFFECT OF A BOOSTER DOSE

	Number. of patients showing the following amount of antitoxin in serum (I.U./ml.)				
	<0.01	0.01–0.1	0.1–0.5	0.5–2.0	> 2.0
Before booster dose	38	27	1	1	3
4–5 days after booster dose	4	5	1		
6–9 days after booster dose	1	2	7	7	9

two to three years after combined active and passive immunization, but only five of them had a satisfactory immunity according to our requirements. They did not show any definite rise in antitoxin content of serum four to five days after the booster dose, but six to nine days after there was a marked rise. In only one case out of twenty-six was the response inadequate.

These investigations show that the combined active-passive immunization procedure has an effect that may still be demonstrated two to three years later by direct titration of the antitoxin content of serum or by the reaction following a booster dose. The antigenic stimulus remains, therefore, even after the disappearance of the passive immunity. In accordance with this finding a single injection of toxoid, even though this be administered together with the usual prophylactic dose of serum, produces a basal immunity, and in the event of another injury a booster dose of toxoid without antitoxin will give adequate protection. Although we do not in any way question the results of Cooke and Jones (1943), who worked with much higher doses of serum, our results show that the dose of serum generally used for prophylaxis does not notably impair the effect of our toxoid.

The basal immunity is also of direct practical importance in the prophylaxis of tetanus following minor injuries which are not treated by a physician. Experience has shown that many cases of tetanus occur after such injuries, and that certain occupations carry a heavy risk in this respect. The primary injection makes a small amount of antitoxin available for a long period. This antitoxin neutralizes the toxin initially produced; the latter acts as a booster dose and causes a further rise in the antitoxin titre. Wolters and Dehmel (1940) showed in man that the quantity of toxin which may be produced after a naturally sustained injury actually has a considerable antigenic effect in an already actively immunized organism. In view of present knowledge a further condition must be stipulated: that there should be no passive immunity present that might interfere with the antigenic stimulus.

Practical Conclusions

Considering the results of the present investigations the following practical rules regarding prophylaxis against tetanus may be recommended.

An injury involving a risk of tetanus in a person not previously actively immunized should be treated with simultaneous active and passive immunization. Even better results would, no doubt, be achieved if the active immunization were not initiated until passive immunity had vanished. Still, since the attention of physician and patient is more easily directed to the prophylaxis against tetanus directly after an injury, simultaneous passive and active immunization is recommended. When, following a severe injury, a higher dose of serum than usual is required, the antigenic stimulus of the toxoid should be protracted so as to exceed the duration of the passive immunity. This protraction may be obtained by a second dose of toxoid or by postponing the first dose of toxoid.

In children prophylaxis against tetanus may be effected by the injection of a mixed tetanus and diphtheria toxoid. This procedure does not give rise to any disagreeable reactions and produces good results (Bigler and Werner, 1941). Any scheme of inoculation that affords good protection against diphtheria will also protect against tetanus arising from small injuries not treated by a physician. A booster dose should be given after major injuries, but serum prophylaxis should never be necessary if the child has once been actively immunized.

Experience from the second world war has served to improve the prophylaxis in military forces during wartime. As a preparatory measure tetanus toxoid may be used in the military forces in peacetime and should be administered at the beginning and at the end of the first compulsory training period, the two injections being thus about one year apart. Booster doses should be given at mobilization and to all battle casualties. Under these circumstances serum should be unnecessary.

Summary

By determining the amount of antitoxin in the serum of patients after the generally adopted dose of 3,000 I.U. of tetanus antitoxin the minimum amount of circulating antitoxin which gives protection against tetanus was estimated. The value found corresponds to 0.1 I.U. per ml. in a man weighing 65 kg.

This level may be achieved by an injection of adsorbed toxoid followed, at the time of injury, by a booster dose of the same toxoid. In man the main effect of the primary injection is not influenced by a simultaneous dose of 3,000 I.U. of antitoxin.

Conclusions are drawn as to the practical application of these experiences in prophylaxis against tetanus.

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THE VALUE OF LIQUOID FOR BLOOD CULTURE

BY

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No single medium has been devised that is suitable for the cultivation of every type of micro-organism from the blood. Penfold and others (1940) confirmed the findings of Elliott (1938) on the superiority of saponin broth for the cultivation of *Strep. viridans*, Hoare (1939) stressed the value of trypsin liver broth in the isolation of anaerobic streptococci from patients with puerperal infections, and more fastidious organisms need even more specialized media. Although these and other recommendations can be accepted, yet the difficulties of putting the various suggestions into practice have not been sufficiently emphasized. Butler (1937) in an important monograph enumerates four different media to be inoculated at the bedside. Penfold and others, while drawing attention to Butler's relatively elaborate technique, themselves recommend four media as a convenient range for routine work. Their methods suggest the need for considerable technical experience and indicate the care required to avoid contamination. The difficulty of maintaining sterility in technique at the patient's bedside is considerable, and is increased by each additional manipulation. In many quarters this has led to the belief that the bacteriologist must actually withdraw the blood as well as carry out the culture. This involves waste of time, even in a hospital, and is impracticable in a public service. Again it is frequently impossible for the bacteriologist to be present at the time most favourable for blood culture. Most of the difficulties arise from the need to distribute the blood into a variety of media immediately after withdrawal. If blood could reach the laboratory in a suitable state, with the infecting organisms unharmed, these troubles would be solved. It seemed likely from the published evidence that the use of liquoid would satisfy these requirements.

Liquoid (Hoffman–La Roche) is sodium polyanethol sulphonate. It has been recognized since 1930 as a powerful anti-coagulant (Demole and Reinert). Battistini (1932) first noted that its property of inactivating complement, thus destroying the bactericidal power of human plasma, made it valuable for blood cultures. Massa and Battistini (1934) used patient's blood treated with 0.16 per cent liquoid as a self-contained culture medium, and claimed excellent results in enteric fever, meningitis, pneumonia, and septic infections. Neisner and Volencová (1934) recommended "liquoid blood" as a transport medium, and many other Continental workers supported the original claim of its usefulness in investigating bacteraemic conditions. Most of these workers used liquoid in a concentration of 0.1 to 0.2 per cent. In this country von Haebler and Miles (1938) presented experimental evidence to show that liquoid in a strength of 0.05 per cent, either alone or incorporated in broth, was excellent for blood culture, but that higher concentrations were occasionally unfavourable. Hoare in general confirmed the findings of these workers but remarked that 0.08 per cent liquoid in liver broth was experimentally inferior to trypsin liver broth for the cultivation of anaerobic streptococci. Penfold and others used liquoid broth and "liquoid blood" parallel with other media in 160 routine blood cultures, and found no evidence to suggest any advantage from their use. Most of their cultures, however, were carried out with liquoid in a final concentration of 0.17 per cent, more than three times that recommended by von Haebler and Miles.

Further information on the rationale of the use of liquoid was provided by Auxilia (1934), who pointed out that, in addition to destroying bactericidal complement, it interfered with the phagocytic activity of leucocytes. Allgöwer (1947) went further, and showed that in experimental tissue cultures traces of liquoid completely prevented phagocytosis, and concentrations as low as 0.01 per cent exerted an obvious inhibitory effect on leucocytic migration.

Accordingly it was decided to use liquoid-treated blood both as a transport medium and as a culture medium and to carry out routine aerobic and anaerobic cultures with small volumes of the blood on various media in addition to incubating it as a self-contained culture. At the same time the results of parallel cultures in an average "enriched" broth medium were to be compared.

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Methods

These were selected as suitable for the large general hospital in which this work was carried out.

Blood-culture outfit.—This consisted of a 10-oz. bottle with a perforated cap and a rubber liner, containing 100 ml. papain digest broth and 5 ml. of sheep's blood (boiled blood broth). To this was attached by a string loop a small 1-oz. (H. 53 U.G.B.) bottle with the screw cap similarly adapted containing 1 ml. of 0.3 per cent liquoid in saline. Both bottles were sterilized by autoclaving. A dry sterilized syringe and two needles were also provided.

Ward technique.—This varied according to the preference of different hospital units. The method recommended was to swab the patient's arm first with spirit-soap or ether-soap, then with surgical spirit. Approximately 10 ml. of blood was taken from the patient and the needle detached from the syringe. The second needle was then fitted to the syringe with a sterile forceps, and 5 ml. of blood was ejected into each bottle by perforating the rubber cap.

Laboratory technique.—Both bottles were incubated for 24 hours except under special circumstances; 0.5 ml. of "liquoid blood" was then distributed by pipette to each of two tubes, one of nutrient broth, one of thioglycollate broth (Brewer, 1940). At the same time a drop of blood was spread on a blood-agar plate and another was smeared on a slide. From the ordinary blood culture bottle subcultures were made to blood agar in the usual way. This routine was re-repeated after a further 24 hours' incubation of the original bottles. Anaerobic plate cultures were carried out as required to confirm the character or establish the identity of organisms seen in films from the boiled blood broth or Brewer's medium but which failed to grow aerobically on solid media. The subcultures and original blood cultures were examined regularly and were not considered negative until after 7 days' incubation. For special examinations the "liquoid blood" was occasionally subcultured to other media, according to indication.

Results

Eight hundred and sixteen blood cultures were made, of which 170, from 141 patients, were positive. The Table shows the relative values of the methods used. The times given indicate approximately the period of incubation before subculture to a solid medium was successful. In most instances the "liquoid blood" inoculum was taken directly from the blood culture bottle, but it was occasionally taken from an early subculture in nutrient or thioglycollate broth.

In the course of this work liquoid was found to have advantages other than the maintenance of blood in a state suitable for diagnostic culture.

Sometimes measured amounts of "liquoid blood" were dispersed in poured plates for the estimation of degrees of bacteraemia, and the blood plasma obtained on standing or by centrifuging was also sometimes used for agglutination tests. Repeated examinations showed no significant difference between the agglutinin titres of serum and "liquoid plasma" from the same source. Thus it was occasionally possible to obtain a serological diagnosis by demonstrating a higher titre in a serum taken for Widal examination than in the "liquoid plasma" of an earlier blood culture. On one occasion a "liquoid blood" culture was obtained from a nurse who had been ill for one week with fever and vague pains in the joints. Four days later her serum Widal test was positive to *S. paratyphi-B* 1/5,000 (H), 1/320 (O), while the titre of the plasma from her earlier blood culture was only 1/640 (H), negative (O). From repeated examinations of similar specimens during periods of incubation the loss in agglutinin titre during such incubation was known to be trifling, and the rise in titre so demonstrated established the diagnosis of paratyphoid fever. It was also possible to obtain an early indication of enteric infection by serological tests on plasma from a "liquoid blood" culture and later to confirm the diagnosis by the isolation of the infecting organism from the same specimen. *S. paratyphi-B* was isolated from such a blood culture with a specific plasma titre of 1/3,200 (H), 1/50 (O). By similar methods it was occasionally possible to determine the aetiological importance of organisms of doubtful pathogenicity. Thus the recovery of *B. coli* from a patient's blood became significant when the "liquoid plasma" was found to agglutinate the organism to a titre of 1/1,280. On three occasions the diagnosis of Weil's disease was suggested by a positive serological test on liquoid plasma before it was clinically suspected.

Discussion

This is the largest series so far reported of blood cultures in which the use of liquoid has been compared directly with more usual methods. "Liquoid blood" has proved to be a good culture medium, but it is now suggested that liquoid is most valuable as a vehicle in which a specimen of blood can be brought to the laboratory for the bacteriologist's investigations. It can then be distributed into various culture media as considered advisable. This answers any criticism suggested by the results of Hoare or of Penfold and others, who used "liquoid blood" itself as a culture medium. Direct trial against a variety of

other recommended culture media has not been attempted, but subculture to digest broth, Brewer's thioglycollate broth, and blood agar appears to allow successful isolation of most of the bacteria likely to be encountered in hospital practice. Penfold and others, using saponin broth, obtained eleven positive results in eleven cultures from patients with *Strep. viridans* infections, figures much superior to those obtained with other culture media used in parallel. In this series forty-six positive *Strep. viridans* cultures out of a possible total of forty-eight were obtained both from "liquoid blood" and from the enriched broth used in parallel. That the failures, one by each method, were probably due to the chance distribution of bacteria in blood specimens, is suggested by the occasional finding that in one blood culture the infecting organism might grow luxuriantly from boiled blood broth and poorly, or not at all, from liquoid, while in a further specimen from the same individual a few days later the reverse would be experienced. During this period of trial no case of subacute bacterial endocarditis due to this infection came to necropsy without a previous diagnosis by blood culture ; and it is unlikely that any established *Strep. viridans* septicaemia remained undetected. It can therefore be assumed that for ordinary aerobic bacteria the use of "liquoid blood" for transport and subculture into the suggested media is as good as the use of other

recommended media, but for certain bacteria it has advantages. In this series anaerobic streptococci, fusiform bacilli, and *F. necrophorus* were isolated more frequently from the "liquoid blood" specimen. This was undoubtedly due to the routine use of a convenient clear anaerobic fluid medium (Brewer's) for subculture. Much heavier inocula can be used than in subcultures from ordinary broth to solid media for anaerobic incubation, with the consequent greater opportunity of recovering such bacteria. Routine anaerobic incubation of subcultures to solid media is also thought in many busy laboratories to be unduly laborious. Early growth in this thioglycollate medium is much more easily seen than in Robertson's meat medium or in liver broth recommended by other workers. Again, an organism such as *L. icterohaemorrhagiae* can never be grown from any ordinary culture medium, yet a small amount of a "liquoid blood" specimen can readily be subcultured to a special leptospiral medium if suspicion of such an infection arises. Although leptospirae were recovered only on one occasion in this series, the general suitability of the procedure has been demonstrated by numerous isolations from the blood of infected guinea-pigs (Stuart, 1943). Since this investigation was completed Allison (1944) has also mentioned the use of liquoid as a vehicle for the transport of blood culture specimens.

TABLE·

COMPARATIVE CULTURE RESULTS FROM BOILED BLOOD BROTH AND "LIQUOID BLOOD"

Organism	No. of patients	Total no. of positive cultures	Boiled blood broth				Liquoid				+ B − L	− B + L
			24 hrs.	48 hrs.	Later	Total	24 hrs.	48 hrs.	Later	Total		
Staph. pyogenes	38	42	22	12	3	37	25	11	2	38	4	5
Strep. pyogenes	20	21	19	—	—	19	18	1	0	19	2	2
Strep. viridans	37	48	23	20	3	46	27	16	3	46	2	2
Strep. non-haemolytic	4	5	2	0	1	3	2	1	1	4	1	2
Strep. anaerobic	3	3	0	0	2	2	0	2	1	3	0	1
Strep. pneumoniae	9	9	8	0	0	8	9	0	0	9	0	1
Micrococcus Sp.	1	4	2	2	0	4	1	2	1	4	0	0
N. meningitidis	1	3	2	1	0	3	2	1	0	3	0	0
H. para-influenzae	2	3	0	1	2	3	0	1	2	3	0	0
S. typhi	2	2	0	1	1	2	0	1	0	1	1	0
S. paratyphi-B	13	16	8	5	2	15	4	6	5	15	1	1
S. enteritidis	1	2	1	0	0	1	0	1	0	1	1	1
B. coli	5	6	6	0	0	6	6	0	0	6	0	0
B. proteus	1	1	1	0	0	1	1	0	0	1	0	0
F. fusiformis	1	1	0	0	0	0	0	0	1	1	0	1
F. necrophorus	2	3	0	1	1	2	0	2	1	3	0	1
L. icterohaemorrhagiae	1	1	0	0	0	0	0	0	1	1	0	1
Total	141	170	94	43	15	152	95	45	18	158	12	18

+ B − L = Positive from broth, negative from liquoid.
− B + L = Negative from broth, positive from liquoid.

TABLE ·

REPRESENTATIVE RESULTS SHOWING THE EFFECT OF TEMPERATURE ON THE LETHAL ACTION OF TOLUENE

Organisms	Temp. °C	Control	Time of exposure to toluene					
			5 secs.	15 secs.	30 secs.	1 min.	2 mins.	3 mins.
Ps. pyocyanea	4	+++	+++	+++	++	++	..0	0
	23·	+++	+++	++	+	12	0	0.
	37	+++	2	—	—	—	0	0·
B. proteus	4	+++	+++	++	+++	+++	+++	++
	23	+++	48	30	—	—	—	—
	37	+++	7 ·	—	—	—	—	—
Diphtheroid bacilli .. ·	37	+++	+++	+++	+++	+++	++	0
Staph. aureus	37	+++	+++	+++	+++	+++	+++	+++
	37	7	0	5	6	9	0	0
Ana robic indifferent streptococci ..	37	+++	+++	+++	+	43	0	0
Str. pyogenes	37	+++	+++	+++	+++	+++	++	0
	37	25	0	37	42	40	0	0

+++ = About 500 to 800 colonies.   ++ = 200 to 500 colonies.   + = 50 to 200 colonies.
48, 42, 40, etc. = Number of colonies grown.   — = Sterile.   0 = Not examined.

and three hundred strains ·of *Ps. pyocyaneus* have been encountered, and a few of these failed to be killed in our standard time of exposure to toluene, but when we obtained a fresh specimen the coliform bacilli were inhibited. Two insensitive strains of each organism were subcultured and each continued to be insensitive, but fresh specimens from the cases concerned contained *B. proteus* or *Ps. pyocyaneus* which were fully sensitive to toluene ; if possible, therefore, when one of these organisms appears to be insensitive a fresh specimen is obtained as the insensitive variants may not be present.

Comparison of the Toluene Technique with the "Fry" (1932) Plate

Sixty-seven specimens containing a mixture of coliform bacilli and Gram-positive organisms were examined by both methods. The toluene technique was simpler and less time-consuming ; the colonies were directly visible and colonial characteristics were unaltered, giving ease of identification and subculture. On the "Fry" plate, colonies—other than haemolytic streptococci—had to be subcultured for identification by pricking through the agar layer, a relatively difficult technique.

The Routine Plate for Wound Swabs

This is a blood agar plate that has a central ditch cut out and removed ; the ditch prevents *B. proteus* swarming across to the toluene-treated side of the plate. Over the outer segment of each half-plate

gentian violet solution is spread (Fleming, 1942). As each batch of' gentian violet varies in its inhibiting power, the optimum concentration must be established experimentally ; our sample needed a dilution of 1/30,000. The gentian violet solution is made up in sterile industrial spirit (Robson, 1945), as it is found that the plates then dry much more quickly than with an aqueous solution of the dye. The plate is dried before planting as there is a tendency for the cultures to become confluent if the gentian violet is spread after inoculation.

1. The swab is first planted on one half of the plate described above and a smear made for a Gram stain. ·

2. The swab is emulsified in 2 to 3 ml. of broth, in a bijou screw-capped bottle ; the bijou bottles are kept ready for use at 37° C.: if there is likely to be an interval before the toluene is added, ⁻the bijou bottle is replaced in the incubator to_prevent cooling.

3. Toluene, approximately one-tenth of the volume of the broth, is added to the emulsion.

4. The bottle is recapped and immediately shaken vigorously for 15 to 20 seconds.

5. With a sterile Pasteur pipette one drop of the suspension is placed on the second half of the blood plate and is then spread in the routine manner ; we do not wait for the toluene to rise and settle out before we remove the broth for planting.

This one plate allows the easy recognition of the relative numbers of the different organisms present. Any coliform bacilli present will grow on the side of the plate planted without treatment by toluene ; if few coliform bacilli are present

they may be swamped by a heavy growth of staphylococci, whereas the use of gentian violet allows them to appear as discrete, recognizable colonies.

The toluene-treated culture will contain no Gram-negative organisms and the relative numbers of Gram-negative and positive organisms will be shown on the two halves of the plate. The part of the plate untreated by gentian violet will show the staphylococci and diphtheroid bacilli present, and alongside this area will be shown the

DITCH

1/30,000 Gentian Violet.

1/30,000 Gentian Violet

TOLUENE— TREATED CULTURE

PLAIN CULTURE
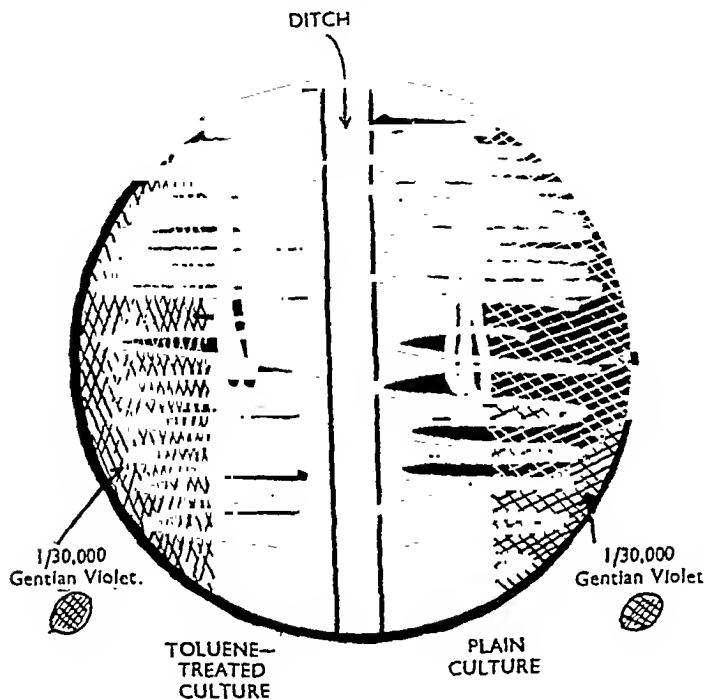
streptococci where the gentian violet has inhibited the staphylococci and diphtheroid bacilli (see Figure).

Faeces.—After the specimen has been planted on the usual special media for Salmonella-dysentery groups, an emulsion is made in broth and this is treated with toluene in the same fashion as a wound swab emulsion : occasionally the coliform bacilli fail to be killed by exposure to toluene for 15 seconds and this may be explained by the work of Benians (1913), who showed that fatty and albuminous substances inhibit the action of toluene.

Urines.—The centrifuged deposit is planted in the laboratory's usual manner, and then some of the deposit is added to the warm broth and treated with toluene as previously described.

Discussion

Many techniques have been evolved to suppress coliform bacilli generally and B. proteus in particular, as their tendency to overgrow other organisms obscures the complete bacteriological picture in mixed infections. Inhibiting agents (phenol, alizarin red, etc.) can be incorporated in the medium, and Bray (1945) exposes the planted plate to ether vapour, but these methods necessitate the use of duplicate plates if the complete picture is to be given.

The 8 per cent agar plate, Hayward and Miles (1943) provides colonies unlike those on ordinary laboratory media, and therefore suffers from the same drawbacks as the " Fry " plate, although the latter was introduced primarily for the isolation of haemolytic streptococci in the presence of B. proteus. Beattie's (1945) method only inhibits the swarming of B. proteus, so if many coliform bacilli are present they may hide small numbers of Gram-positive organisms.

Toluene appears to inhibit the growth of all pathogenic organisms if it is left long enough in contact with them, the inhibiting action being accelerated by increased temperature. The Gram-negative organisms are very susceptible to its action, whereas the Gram-positive organisms are relatively resistant. We now employ toluene in the primary cultivation of any specimen that is likely to contain coliform bacilli ; we use it on all wound swabs and faeces, and in some cervical, urethral, and aural swabs, urines, post-mortem material, and sputa.

Summary

A method of inhibiting coliform bacilli in cultures is described : also a new method of applying gentian violet in a composite plate which gives a complete and unobscured bacteriological picture on the one culture.

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TECHNICAL METHODS

A MACHINE FOR WASHING WASSERMANN TUBES

BY

E. A. ATKINSON

One of the most irksome tasks in a pathological department is the washing of test-tubes. Particularly is this so when a thousand or more tubes have to be washed at the end of a long day doing Wassermann and Kahn tests. If the task is postponed to the next day the cleaning process becomes more difficult. It seemed that a machine was needed for the mechanical rinsing of tubes immediately after the conclusion of the tests. The apparatus to be described has been in use in this laboratory for several months and serves as an efficient rinsing machine.

Description of Apparatus

Test-tubes are contained in revolving circular racks sprayed from below by a system of water jets. The racks are 7 in. in diameter and have bottoms of 18-gauge copper wire mesh, six to the inch. Each rack is divided into four compartments by partitions radiating from a central hollow shaft. The jets are thirty or so in number, and so arranged as to spray a sector of the bottom of the rack immediately above. One row of jets is inclined at an angle—this provides a lateral thrust and maintains a slow revolution of the rack.

Method of Use

A rack is filled with tubes placed upside down (280 $\frac{3}{8}$-in. tubes or 160 $\frac{1}{2}$-in. tubes) and the glass cover (an inverted mouse jar) is placed in position. The tubes are sufficiently rinsed after the water has run for two or three minutes, when another full rack is placed in position and the process is repeated. It is advisable to have at least half a dozen racks. The racks are partly drained and then placed in the hot air oven. Given a sufficient number of racks, the tubes may be left in position until required again.

The method is not suitable for very dirty or greasy tubes requiring drastic chemical treatment, but is suitable for large-scale mechanical rinsing. In my experience the tubes emerge bright and clean from the drying oven in spite of a very hard water supply.
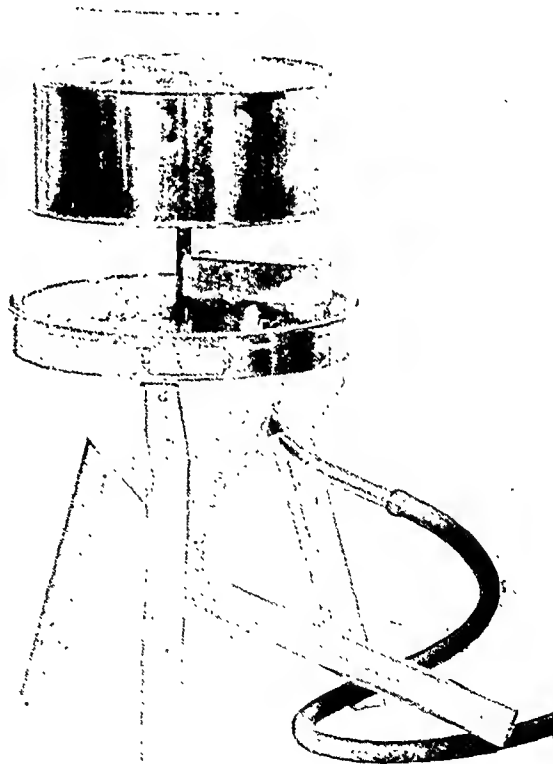
The machine illustrated was made by Messrs. Jackson, of 348, City Road, London, E.C.1.

A MACHINE TO ASSIST IN RHESUS-GROUPING

BY

E. A. ATKINSON

The routine examination of blood during the antenatal period for the rhesus factor has become an important function of many hospital laboratories. The area served by this department provides more than 10,000 such specimens a year, and it was important to find a method of performing the agglutination test with anti-D serum involving as little effort or chance of confusion as possible. The device to be described employs the tube test, and the reading of the results is simplified by the projection of a small lantern image of the red cell sediment on a screen.

Description of Apparatus

The machine consists of a circular rack revolving round a vertical axle. Above is suspended a dark-

FIG. 1.—The rack

ground illuminator: below is fixed a small lantern projection lens. The rack consists of two circles of one-eighth gauge steel plate (13 in. diameter) drilled to take two concentric rings of $\frac{1}{2}$-in. × 3-in. tubes with a bottom plate of $\frac{1}{4}$-in. plate glass. All three are joined to a hollow-threaded central shaft. The compound projection lens is made of two biconvex lenses, each 3 cm. diameter and of 6 cm. focal length and placed directly under the outer ring of tubes. The holes are numbered to take 32 tests.

Method of Use

The inner row of tubes is used for the preparation of the washed red cell suspensions. The outer circle of holes is then loaded with the reaction tubes. One large drop of red-cell suspension is then transferred from each inner tube to the corresponding reaction tube. A very small drop of anti-D serum is then added to each reaction tube and the whole rack is placed in the incubator. After periods of one and two hours the rack is placed on the axle and readings are taken. In actual practice the positive results are usually clearly readable after about half an hour.

Advantages Claimed

The machine is useful for the mass testing of routine specimens. As with the Chown method, rapid settling out of agglutinated particles occurs, but with rather less economy of serum. At the same time, the advantages of a tube test are retained. By the device described, the reading of the result of the tube test is simplified, and the lantern images correspond well with the microscopic findings.
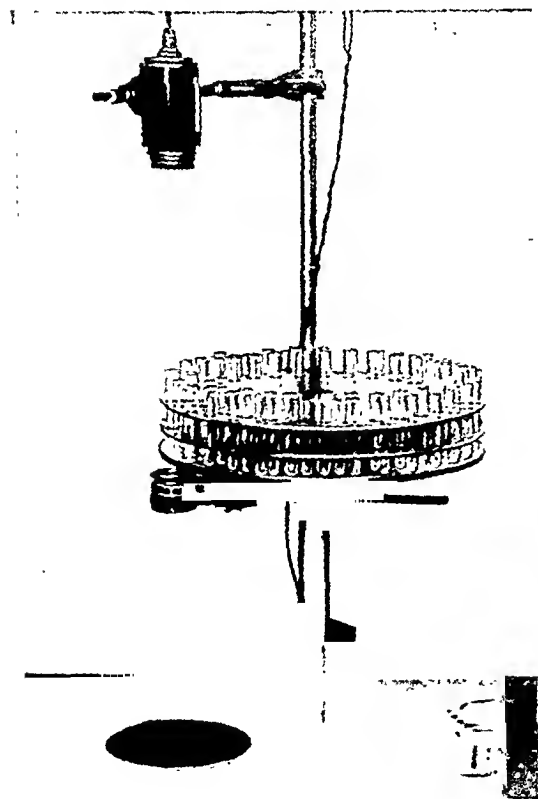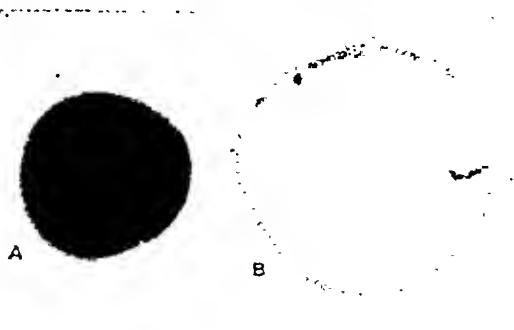
FIG. 2.—(a) Negative result. (b) Positive result after one hour's incubation.

OBITUARY

GORDON WILKINSON GOODHART

Dr. G. W. Goodhart, M.D., F.R.C.P., Bunny Good-hart as most of his friends liked to think of him, died in London on July 16 just before his 66th birthday. He was the younger son of Sir James Goodhart, a consulting physician in the great Victorian tradition. He was educated at Westminster School, Trinity College, Cambridge, and Guy's Hospital, graduating M.B. in 1908. At Guy's he became successively house-physician, Gull research student, Douglas demonstrator in pathology, and senior assistant bacteriologist. Subsequently he worked in Berlin, and for a time with Aschoff at Freiburg, returning to become clinical pathologist at University College Hospital. In the 1914–18 war he was in France with the R.A.M.C., and served as pathologist to the Second London General Hospital. In the following years at University College Hospital, apart from teaching students and his own junior staff, he carried on continuous research with Charles Bolton on gastric secretion and constantly helped and stimulated the fundamental work of Price Jones on red cell size. In 1931 he was appointed as one of the first four pathologists to the London County Council, and he did much to establish and develop their highly success-ful service. He worked first at the Archway Group Laboratory and then at St. Mary Abbots. He was in due course President of the Section of Pathology of the Royal Society of Medicine, of the Medical Society of London, and of the Association of Clinical Pathologists. To this Association in its early years he gave unfailing help and encouragement.

Physicians and surgeons were always to be found in Goodhart's laboratory at University College Hospital, or later in his London County Council laboratories, discussing their problems with him. He himself was constantly to be seen in the wards looking at the patients. The knowledge he gained in his laboratory only became real to him in relation to the individual patient. Case reports or pathological reports alone were meaningless and both must be interpreted in relation to the actual human being. So he taught a succession of students, assistants, and colleagues, for from his early days at U.C.H. all medical students served a three months' clerkship in his department. This, though now an accepted procedure in medical

GORDON WILKINSON GOODHART

training, was an innovation, part of Goodhart's contri-bution to the medicine of his time. Many of his pupils practising in very different fields to-day when they meet agree that Bunny Goodhart taught them what really mattered in their medicine. His original contributions to knowledge were all sound, but so much of what he had learnt by careful observation

and experience he never wrote down but handed on in his day-to-day teaching and conversation. This is especially true in the expanding field of haematology.

During the last five years he was often very ill, but he carried on with a courage and gaiety that will always be an inspiration to remember. His hospital might quite literally be brought down on top of him, but there he still was ready to help both with medical and personal problems. Still finishing the last story, he saw one out over the heaps of rubble just as in safer days he had seen one down the laboratory stair. Those long stories were very much part of the man: they were never unkind, they were often shrewd, and he told them with such evident enjoyment himself that the listener enjoyed them too.

Gordon Goodhart had a fine mind but no great ambition to achieve academic or worldly distinction. He was content to do his day's work well, to return after tea to an exceedingly happy home with his wife and their three children, to keep alive a host of friendships; but when the medical history of the early twentieth century comes to be written he will be remembered as one of the leaders of a small group of men who, because of their own skill as physicians combined with their love and understanding of the more precise discipline of the laboratory, created that hybrid despised by some but none the less essential to the developing medical sciences—the clinical pathologist. His friends to-day miss him and will remember him as, in the fullest sense of the words, "a beloved physician."

JANET VAUGHAN.

REVIEWS

Pathologische Anatomie und Chemotherapie der Infektionskrankheiten. By Gerhard Domagk. Stuttgart: Georg Thieme Verlag. 1948. Pp. 424. 133 illustrations. Price, bound, D.M. 48.

Hardly more than a generation has passed since British medicine looked to Germany for inspiration and for advanced books, perhaps in no branch of medical science more than in the field of pathology. English textbooks are now as eagerly sought by medical students in most parts of the world. Nevertheless a new publication in German still evokes the interest of British students and research workers, especially one from the pen of Professor Domagk, whose name is so closely associated with pioneering research on the sulpha drugs. The general plan of relating the action of the antibacterial drugs to the pathological process of infection with a detailed study of the tissue changes is a good one and is well done. The student will also benefit from the author's classification of infections from a pathogenetic point of view, although this classification should not be regarded as rigid. The disturbing feature of the book is that, although the title suggests a wider field, in fact only bacterial diseases are dealt with and even they are not fully covered. The reader will progressively come to realize

that the term "infectious disease" is here used almost synonymously with those infections which are affected by the sulpha drugs. The book is, in fact, a survey of sulphonamides and covers a wide field of research into their uses and actions: as such it is an excellent reference book. The author may naturally have stressed his own particular interests, but the need for full information on all modern antibiotics and their uses and limitations is urgent, particularly for German doctors who have had till now such slight experience of penicillin, streptomycin, etc., and who may be given a one-sided impression by this book. It is to be hoped that this deficiency will be remedied in the next edition.

H. G. KOHLER.

British Drug Houses have produced a pamphlet entitled "Introductory Notes on Chromatography," which they are prepared to send without charge to clinical pathologists who may be interested in this subject. The monograph is clearly meant to cover as wide a range as possible. Those interested in the specific aspect of the field will find the bibliography useful.

NICHOLAS MARTIN.

ABSTRACTS

This section of the JOURNAL *is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this* JOURNAL *some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.*

BACTERIOLOGY

The Recognition of Toxicogenic Bacterial Strains *in vitro*. ELEK, S. D. (1948). *Brit. med. J.*, 1, 493.

This *in vitro* test for the detection of toxicogenic strains of various organisms is based on the principle of floccula-tion of a toxin-antitoxin mixture. In order to establish optimum conditions for the latter, the author pours plates and embeds, while the medium is still soft, a strip of filter paper previously dipped into specific antitoxin. After the medium solidifies the plates are inoculated by streaks at right angles to the filter paper. The toxin produced by the subcultures and the antitoxin contained in the filter paper diffuse into the medium, and after 24 to 48 hours precipitation occurs in a line and at an angle of about 45 degrees at either side of the inoculum, thus appearing as an arrow-head. Technical details are given and non-specific reactions are discussed. The method appears to be particularly suitable for the detection of virulent strains of *Corynebacterium diphtheriae* and, in the hands of the author, yielded results identical with those of the guinea-pig test. *R. Sahn.*

Post-examination of BCG-material. (In English.) TÖRNELL, E. (1947). *Acta tuberc. scand.*, 21, 241.

The author reviews the results of BCG vaccination as carried out at, or in connexion with, the Dispensary in Borås, Sweden, run by the Västeråsen Sanatorium. Between 1935 and 1945 over 10,000 persons were vaccinated, including child and adult contacts, school-leavers, factory-workers, and army conscripts. From 1946 onwards all newborn babies in the maternity homes were also vaccinated. Technique and isolation precau-tions are described in detail. The dose of vaccine used has gradually been increased over the years from 0.05 mg. to 0.06 to 0.08 mg. Wallgren's intracutaneous method was employed, and a weal 10 to 12 mm. in diameter was aimed at.

Out of 10,963 individuals only 17 developed tuber-culosis later; 12 of these, who had been exposed to infection before vaccination was completed, fell ill within 3 years of vaccination; but the other 5 became ill after 4 years. Full and careful follow-up and records of case histories are given, with 119 unvaccinated tuber-culin-negative relatives acting as controls.

Allergy and immunity do not always run parallel. Although allergy fades out in about 4 years, a changed level of reaction does persist, indicating a certain degree

of immunity. Nevertheless, all vaccinated persons who are tuberculin-negative after 4 years should be revac-cinated and persons who react only weakly should be retested each year.

p-Aminosalicylic Acid in the Chemotherapy of Tuber-culosis. (*p*-Aminosalicylsäure in der Chemotherapie der Tuberkulose.) RAGAZ, L. (1948). *Schweiz. med. Wschr.*, 78, 332.

There was rapid improvement in 60 to 70% of pul-monary tuberculosis, with amelioration of all the clinical signs, when treated with 10 to 15 gr. of *p*-aminosalicylic acid daily on alternative weeks. A 10% solution of the drug may be employed in the local treatment of tuber-culous empyema and abscesses. It has so far failed to cure patients with tuberculous meningitis and miliary tuberculoses, but clinical experience is still too small to permit critical assessment of its value.

Streptomycin Resistant Tubercle Bacilli. Their Develop-ment during Streptomycin Therapy of Pulmonary Tuberculosis. FISHER, M. W. (1948). *Amer. Rev. Tuberc.*, 57, 53.

Sensitivity of Tubercle Bacilli to Streptomycin. An *in vitro* Study of some Factors Affecting Results in Various Test Media. FISHER, M. W. (1948). *Amer. Rev. Tuberc.*, 57, 58.

The sensitivity to streptomycin of tubercle bacilli from the sputa of 20 patients suffering from pulmonary tuberculosis, and receiving 1.8 g. streptomycin daily for 120 days, was studied, the Dubos albumin-"tween 80" medium being used. In 15 patients the bacilli remained sensitive. In the remainder sensitivity was retained for up to 11 weeks, after which varying degrees of resistance developed. Since this finding contrasted with that of Youmans *et al.*, who found that resistance developed in 8 out of 12 patients, strains isolated at the end of therapy from the 20 patients of the present series were compared by sensitivity tests in Dubos medium and in Youmans' medium (which contains 10% human plasma and 2% glycerin). Eleven of the 20 strains were resistant in the Youmans' medium (growing in 10 µg. or more of strepto-mycin per ml.).

The factor responsible for these discrepancies was found to be the "tween 80" (oleic acid ester). In the Dubos basal medium alone (without albumin or tween) all these strains were resistant to 1,000 µg. streptomycin per ml. Addition of albumin made no difference, but if

tween 80 was added (without albumin) the strains were sensitive to 1 μg. per ml.—that is, the effect of streptomycin was enhanced 1,000-fold. When albumin as well as tween was added the sensitivity was still increased but not as fully as when tween 80 was used alone. The potentiation of streptomycin by tween 80 may be due to surface-activity causing easier absorption of the antibiotic.

Streptomycin Treatment in Intestinal Tuberculosis. (Die Streptomycinbehandlung der Darmtuberkulose.) MARKOFF, N. (1948). *Schweiz. med. Wschr.*, 78, 329.

A preliminary report is given of the beneficial effect of streptomycin in the treatment of 5 cases of ulcerative intestinal tuberculosis. The streptomycin was administered parenterally and by means of high enemata, in a strength of 250 mg. per 500 ml. of normal saline. Daily enemata are given for 14 to 15 days and are well tolerated. By elevating the pelvis and placing the patient on the right side it is possible to make the solution pass through the ileocaecal valve. A 6-month survey showed that there was invariably an improvement in the radiological findings, a decrease in the toxaemia, and a general subjective improvement. Complete regression of the intestinal lesions has not yet been observed. The results so far are, however, eminently satisfactory, considering the hopelessness of the condition. Streptomycin in intestinal tuberculosis must, however, be given in a hospital or sanatorium where the pulmonary and general condition can be accurately assessed. *Harold Jarvis.*

Recovery of Streptomycin from Urine. MILLER, J., and ROWLEY, D. (1948). *Lancet*, 1, 404.

In the treatment of tuberculous patients with streptomycin it was found that about 50% of the drug was excreted in the urine at a concentration of about 1,000 units per ml. The streptomycin could be recovered from the urine, by simple adsorption methods, in a sufficiently pure form for reinjection, thus effecting a saving of the drug during the present shortage. *R. Wien.*

Streptomycin in the Treatment of Tularemia. BENSON, R. C., and HARWELL, A. B. (1948). *Amer. J. med. Sci.*, 215, 243.

The authors summarize the clinical data in 56 cases of tularemia treated by streptomycin; 15 were examples of pleuro-pulmonary tularemia, in 10 of which the primary site of infection appeared to be the lung or pleura. The authors point out that to be beneficial streptomycin must be administered before the twelfth day of illness, otherwise it will not prevent the breaking down of infected lymph nodes. Streptomycin therapy was highly efficacious in modifying the course of the disease in every case, being most effective in the severest cases. Duration of treatment varied from 4 to 18 days, averaging 9.1 days. The total dosage was from 1.9 to 20 g., averaging 8.1 g. Only 3 patients manifested any intolerance, but in none was it necessary to discontinue treatment.
Jos. B. Ellison.

Streptomycin in Surgical Infections. IV. Peritonitis. PULASKI, E. J., SEELEY, S. F., and MATTHEWS, C. S. (1947). *Surgery*, 22, 889.

A clinical s udy was carried out on young men who were in good health before the onset of peritonitis. In addition to streptomycin the majority of the patients had full supportive therapy. The authors conclude that streptomycin has a place in the treatment of peritonitis,

results of therapy by this agent alone running approximately parallel to those with large doses of penicillin. Streptomycin appeared to have little effect in localized infections but was useful in a spreading peritonitis, effecting localization in such cases. Used with penicillin, it was effective in many cases which failed to respond to penicillin with or without sulphonamides.
A. E. Porritt.

Streptomycin in Human Plague. KARAMCHANDI, P. V., and SUNDAR RAO, K. (1948). *Lancet*, 1, 22.

Five moribund patients, all proved by gland puncture to be suffering from plague during an epidemic in the Madras area, made good recoveries. Improvement started on the second day after about 1.5 g. of streptomycin had been given; 4 g. was a sufficient total dose when given 3-hourly in intramuscular injections of 0.125 g. *C. C. Chesterman.*

A New Sulpha Compound ("6257") and Its Use in Human Cholera Infection. BHATNAGAR, S. S., FERNANDES, F., DE SA, J., and DIVEKAR, P. V. (1948). *Nature, Lond.*, 161, 395.

Chemotherapy of Cholera with a New Sulphonamide Compound ("6257"). Laboratory Investigations and Field Trials. BHATNAGAR, S. S., FERNANDES, F., DE SA, J., and DIVEKAR, P. V. (1948). *Brit. med. J.*, 1, 719.

Hexamethylene tetraamine was found to kill cholera vibrios suspended in physiological saline in less than half an hour. Crude preparations consisting of sulphanilamide and hexamines were first prepared and found to give promising results in animals and man infected with cholera. Later a compound ("6257") was prepared by condensing two molecules of sulphathiazole and three molecules of formaldehyde. *In vitro* it exerts a bactericidal action on cholera vibrios when 50 mg. or more is added to 10 ml. of peptone water; in concentrations of from 5 to 50 mg. in 10 ml. it is bacteriostatic. *In vivo* in mice inoculated intraperitoneally with cholera vibrios complete protection is afforded by doses of 40 to 50 mg. given subcutaneously or intraperitoneally for 2 days before inoculation and for 4 days after. If given by mouth only 10% of mice are protected, probably because of poor absorption from the alimentary canal.

Field trials were carried out in villages in Southern India. After bacteriological confirmation of the diagnosis a dose of 6 g. was given by mouth followed by 4 g. 4 hours later. Two doses of 2 g. were given on the second and third days, two doses of 1 g. on the fourth day, and 1 g. for the next 3 days. The usual total dose was 28 g., though as much as 50 g. has been given without any toxic results. If vomiting was severe the first doses were given by rectum. Among 85 patients, mostly undernourished women and children, the mortality has been 4%, whereas in the same area the average mortality for the past seven years has been 70%. Although no details are given the administration of the drug to healthy contacts is said to have protected them against infection.
G. M. Findlay.

Treatment of Epidemic Typhus with Chloromycetin. PAYNE, E. H., KNAUDT, J. A., and PALACIOS, S. (1948). *J. trop. Med. Hyg.*, 51, 68.

Chloromycetin is an antibiotic obtained by Ehrlich *et al.* (*Science*, 1947, 106, 417) from an actinomyces isolated from the soil of a field in Venezuela. It has been found

by Smadel and Jackson (*Science*, 1947, 106, 418) to be active against psittacosis virus and against a number of rickettsiae in mice. It has now been used on 16 patients in Bolivia with epidemic - louse-borne typhus. The antibiotic may be given intravenously or by mouth, the former giving more rapid results. Intravenous medication consists of doses of 10 mg. per kilo body weight for 3 days, while by mouth at least 15 mg. per kilo of body weight may be given without toxic reactions for the same period.   *G. M. Findlay.*

Observations on the Action of Paludrine on Malarial Parasites. MACKERRAS, M. J., and ERCOLE, Q. N. (1947). *Trans. R. Soc. trop. Med. Hyg.*, 41, 365.

When "paludrine" was given to patients infected with *Plasmodium vivax* a lethal action was exerted upon the dividing nucleus of the malaria parasite, which is rapidly destroyed. Paludrine does not directly prevent the formation of gametocytes or destroy those already formed; it does not prevent fertilization in the gut of the mosquito. Similar effects are observed with *P. falciparum*. In the presence of paludrine gametocytes can be formed; they persist in the blood, undergo fertilization in the mosquito, and reach the stage of encystment, but development ceases at that point. Paludrine exerts a similar effect on the early schizonts of *P. malariae*, although these are somewhat less sensitive than those of the other two species of malaria.   *F. Hawking.*

Bacteriology and Biology of Disinfection of Bile, with Special Reference to Pyridine-3-carbonyl-hydroxymethylamide (Zur Bakteriologie und Biologie der Gallendesinfektion, unter besonderer Berücksichtigung des Pyridin-3-karbonsäureoxymethylamids). ACKLING, O. (1948). *Praxis*, 37, 65.

Pyridine-3-carbonyl-hydroxymethylamide ("bilamid") is a new chemical compound which can be regarded as nicotinic acid amide linked to formaldehyde.

Nicotinic acid plays an important part in the metabolism of the liver, and this new compound exerts its action on the liver and bile. The nicotinic acid component promotes the normal metabolic activities of the liver cells while the formaldehyde component acts as a disinfectant on organisms present in the bile ducts and gall-bladder. The results of animal tests are given showing that when given intermittently for several consecutive days a total daily dose of 2 g. per kilo causes no symptoms, while 4 g. per kilo represents the minimal lethal dose for this scheme of medication. Maximal concentrations of bilamid in the bile are obtained 2 hours after an oral dose of 2 g. per kilo or half an hour after 4 g. per kilo. Within the following 4 to 6 hours the level falls rapidly but traces are still present after 24 hours. Excretion, therefore, is rapid and complete. The drug is also excreted in the urine and can be demonstrated there 1 hour after an oral dose of 4 g. per kilo. The urine content steadily increases until 3 to 4 hours after administration; no bilamid is present in urine after 10 hours.

Bilamid has a bactericidal action on most strains of the coliform group, including organisms causing enteric fever, on *Streptococcus faecalis* and *Pneumococcus* strains, on *Brucella abortus*, and on the cholera vibrio. Bacteriostatic but not bactericidal actions of bilamid were recorded for dysentery, coli, anthrax, and pyocyaneus bacilli as well as for staphylococci.

Figures and tables are given to demonstrate the action of the drug on the flora found in specimens of bile.
           K. Zinnemann.

The Treatment of *B. coli* Urinary Infections with Sulfathalidine (Phthalylsulfathiazole). EVERETT, H. S., VOSBERG, G. A., and DAVIS, J. M. (1948). *J. Urol.*, 59, 83.

As the result of a controlled series of cases the author has concluded that phthalylsulphathiazole is an effective drug in the treatment of urinary tract infections due to *B. coli*. The urine is usually rendered sterile in 1 week by the administration of 0.1 g. per kilo body weight daily. Experience has shown that continuation of the treatment for an additional 2 weeks gives greater protection against recurrence. The author suggests that the good results are due mainly to the action of the drug in eliminating in the bowel the source of infection of the urinary tract.
           Thomas Moore.

Observations on the Prevention of Bacterial Growth by Sulphonamides, with Special Reference to the Harper and Cawson Effect. WALKER, N., PHILIP, R., SMYTH, M. M., and McLEOD, J. W. (1947). *J. Path. Bact.*, 59, 631.

The phenomenon of inhibition of staphylococci and streptococci by horses' blood in an area adjacent to a sulphathiazole trench, known as the Harper and Cawston effect (*J. Path. Bact.*, 1945, 57, 59), has been more fully investigated by the present authors in an endeavour to find an accurate explanation. Many types of blood have been examined: only asses' blood has anything like the same power as horse blood. Beyond revealing the fact that the property appears to be in the haemoglobin fraction, further analysis has not been helpful.

The Problem of Sulfonamide-resistant Hemolytic Streptococci. HARTMAN, T. L., and WEINSTEIN, L. (1948). *New Engl. J. Med.*, 238, 560.

The authors have studied 167 patients suffering from Group A haemolytic streptococcal infections. Most of the cultures were isolated from the nasopharynx of patients who had clinical scarlet fever. The type distribution of these haemolytic streptococci and their sulphonamide sensitivity were studied. Only one strain, a Group A, Type 19, was resistant to the action of sodium sulphadiazine, and was so in a concentration of 25 mg. per 100 ml. The epidemic Type 19 strains prevalent among Service personnel were resistant to a similar concentration of sulphadiazine.
           R. N. Johnston.

Nose and Skin Carriage of *Staphylococcus aureus* in Patients Receiving Penicillin. MOSS, B., SQUIRE, J. R., and TOPLEY, E. (1948). *Lancet*, 1, 320.

Numerous publications have described the presence of *Staphylococcus aureus* in the nasal cavities of a high percentage of healthy people. The presence of the same organism, defined by a positive coagulase test, is of less frequent occurrence on the healthy skin. The authors in their present study have concentrated on the specific problem of the dependence of skin carriage on nasal carriage in persons with normal skins. Some of the patients in a male surgical ward were the subjects of the investigations over a period of 8 months. Penicillin was given intranasally for 10 days: (1) by spraying thrice daily with a solution containing 12,500 units per ml., and (2) by applying a cream containing 100,000 units per g. in a "Lanette wax" base with sterile swabs twice daily. The details of the bacteriological technique are given.

In a group of 21 patients the nasal-carriage rate fell from 97% before treatment to 67% during the first 5 days of treatment and to 37% during the last 5 days, whereas in a control group of 20 the bacterial flora of the nose showed no significant change as a result of their stay in hospital. The elimination of the nasal *Staph. aureus* was associated with a significant fall in its carriage on the skin of the wrist, and this would seem to show that the skin is often contaminated from the nose. In 37 cases swabs were obtained from the vestibule, nasal fossae, and nasopharynx, and the findings emphasize the decreasing frequency with which the staphylococcus is found at sites from the nasal vestibule to the nasopharynx, and that colonization seems to take place only in the squamous epithelium of the vestibule. In 15 cases parenteral administration of penicillin did not affect the incidence of staphylococci in the nose. *J. Smith.*

Penicillin Therapy in Diphtheria. (In English.) WSZELAKI, S. J., and HANDZEL, L. J. (1948). *Acta med. scand.*, 129, 493.

The authors' proposals for treatment are as follows: (a) In the early case penicillin alone may be given, if there is adequate opportunity for clinical observation of the course of the illness. Penicillin may also be used alone where serum treatment is contraindicated—the authors list as examples, active pulmonary tuberculosis, hypersensitization, and "grave blood diseases." (b) Combined penicillin and serum treatment is indicated in a penicillin-sensitive concomitant infection, in very severe but uncomplicated diphtheritic infections and late cases, and in cases "refractory" to serum treatment. A sufficient dose of penicillin is 100,000 units a day.

[It may be noted that simple penicillin treatment in certain cases of diphtheria has been recommended by De, Chatterjee, and Ganguli (*Brit. med. J.*, 1947, 1, 376) and strongly condemned by Long (*Brit. med. J.*, 1947, 1, 884), who has suggested that 1,000,000 units of penicillin daily may be advantageously combined with serum treatment in severe cases.] *G. J. C. Ingram.*

Effect of Penicillin on the Bactcremia Following Dental Extraction. GLASER, R. J., DANKNER, A., MATHES, S. B., and HARFORD, C. G. (1948). *Amer. J. Med.*, 4, 55.

The authors report their observations in 40 patients who received 50,000 units of penicillin intramuscularly 2-hourly for 24 hours before the extraction, and compare the results with those obtained in controls who did not receive penicillin. Blood for culture was taken before and usually within 2, but always within 5, minutes after the extraction. Penicillin reduced the incidence of bacteriacmia considerably, but failed to prevent it in all cases; the organisms grown were in both series α-haemolytic streptococci and non-haemolytic streptococci. The difference between the penicillin series and the control series was greatest in the groups with diseased gums, where the incidence of bacteriacmia was 41.4% (penicillin) and 78.9% (controls). A pronounced difference in favour of penicillin was also seen in the group of single extractions; with multiple extractions the percentages were equal in both series. Local analgesia was used in all cases; the effect of penicillin was greater, comparatively, in the group who had infiltration analgesia than in those who had block analgesia. The

authors recommend that patients suffering from valvular or congenital heart disease should receive large doses of penicillin for 24 hours before and at least 2 days after extractions, which should preferably be carried out singly. *B. Samet.*

Penicillin in the Treatment of Actinomycosis. NICHOLS, D. R., and HERRELL, W. E. (1948). *J. Lab. clin. Med.*, 33, 521.

The authors record the results of treatment of 46 cases of actinomycosis with penicillin. The dose varied from 80,000 to 1,000,000 units of penicillin daily (intravenously or intramuscularly) for periods of from 2 to 7 weeks. They conclude that a dose of at least 500,000 units should be administered daily for at least 6 weeks. "Penicillin appears to be an effective chemotherapeutic agent in the treatment of actinomycosis and a useful adjunct to other forms of therapy." *Zachary Cope.*

Penicillin Preparations with Delayed Excretion in Ophthalmology. (La penicilina de eliminación retardada en oftalmologia). MATA LOPEZ, P. (1947). *Arch. Soc. oftal. hisp.-amer.*, 7, 1111.

The disadvantage of penicillin is its rapid excretion; a further disadvantage in ophthalmology is that penicillin, when given intramuscularly, does not penetrate into the posterior parts of the eye. Various methods have been devised for delaying excretion of the drug, such as combining penicillin and blood plasma, cooling the site of injection, and using a wax-oil base for the penicillin. The author has found this last method most satisfactory and has used 3 such types of preparation, 100,000 units being given daily in two doses, one at night and the other in the morning. As an alternative an aqueous solution of penicillin may be given 3-hourly during the day and the emulsion of penicillin at night.

The author uses penicillin for persistent staphylococcal infections of the eyes, abscesses of the lids, dacryocystitis (local and general injections), gonococcal infections of the conjunctiva and cornea (combined with local application), infections of the cornea, certain cases of episcleritis, and uveitis. He has had good results with this method, which he has also employed prophylactically in cases of trauma. *E. E. Cass.*

Bacteriologic Follow-up of Penicillin-treated Gonorrhea in Women. HIRSCHBERG, N. (1948). *Amer. J. Syph.*, 32, 141.

The author took the opportunity of observing the results of penicillin therapy over a post-treatment period of 10 weeks in women imprisoned for prostitution. Cases were selected for study only after the isolation and identification of the gonococcus by culture and fermentation tests with specimens from the urethra and cervix. All 54 cases were treated with 150,000 units of penicillin in oil-wax. There were only 2 failures of this treatment. In one the gonococcus was found again 3 weeks after treatment, and again 2 weeks after a second treatment. In the other case a positive culture was not obtained until 7 weeks after treatment. The author considers that cultures at regular intervals after apparent cure are of considerable importance. He also comments upon the larger number of Gram-negative bean-shaped diplococci which might be confused with the gonococcus in stained smears or in cultures when the results are unconfirmed by fermentation tests. *V. E. Lloyd.*

Mycotic Vulvovaginitis and the Vaginal Fungi. A Report of 280 Patients. JONES, C. P., CARTER, B., THOMAS, W. L., ROSS, R. A., and CREADICK, R. N. (1947). *Amer. J. Obstet. Gynec.*, 54, 738.

The authors state that there are three groups of yeast or yeast-like fungi which may be found in the vagina—*Monilia*, *Cryptococcus*, and *Saccharomyces*. These organisms can only be distinguished by the morphological characteristics of colonies grown on special media. *Monilia*, or *Candida* (the name now recommended), is probably the only organism of this type which gives rise to symptoms. The clinical findings and diagnosis are described, and the methods of collecting and culturing specimens from patients are given. Intradermal skin tests and agglutination reactions are of little value in diagnosis. For treatment, a vaginal jelly with a "bentonite" base containing calcium and sodium propionate has given excellent results. *Braithwaite Rickford*.

Cultural and Serologic Studies on Granuloma Inguinale. DUNHAM, W., and RAKE, G. (1948). *Amer. J. Syph.*, 32, 145.

After many unsuccessful attempts to grow *Donovania granulomatis* on artificial media, material from the ninety-second egg passage was successfully cultivated by incubation at 35° C. for 1 to 2 weeks after planting on slants consisting of 10 ml. of beef-heart infusion agar with 1 ml. of normal yolk from six-day embryos. Eight serial transfers were made with this medium and also to slants prepared from 5 ml. of 3% agar in tryptone beef-heart infusion broth and 5 ml. of modified Levinthal's stock broth. Colonies were irregular in outline, shiny, translucent, and grey. An antigen was prepared for serological studies by washing and suspending in M/100 phosphate buffered physiological saline, shaking and centrifuging, and employing the supernatant fluid, which was preserved in 1 in 10,000 "merthiolate." When complement-fixation tests were carried out with this as the antigen, 50 positive reactions were obtained from 58 sera of patients with granuloma inguinale, from 1 of 32 with syphilis, from 2 out of 18 with gonorrhoea, from 2 of 7 with lymphogranuloma venereum, from 3 of 10 with chancroid, and from 4 of 19 with varicose ulcers. No positive reactions were obtained from the sera of 10 patients with tuberculosis or of 4 normal persons. *R. R. Willcox*.

Cardiolipin Antigens in Serologic Tests for Syphilis. GIORDANO, A. S., CULBERTSON, C. S., and HIGGINBOTHAM, M. W. (1948). *Amer. J. clin. Path.*, 18, 193.
Cardiolipin Blood Tests in Syphilis. ANDUJAR, J. J., ANDERSON, M. M., and MAZUREK, E. E. (1948). *Amer. J. clin. Path.*, 18, 199.

The theme of these articles is that cardiolipin is an advance on all other antigens, but that the best use has not yet been made of it. In the first paper 24,085 tests were examined, and in the second paper 24,609. It is concluded that cardiolipin antigens are highly sensitive and satisfactorily specific, and have the advantage of being stable and yielding a reproducible emulsion. Sponsored universal tests based on cardiolipin are urged.

The Incubation Period of Ophthalmia Neonatorum. SORSBY, A. (1947). *J. Obstet. Gynaec. Brit. Emp.*, 54, 842.

The principal causal organism in ophthalmia neonatorum is now considered to be *Staphylococcus aureus* rather than the gonococcus, although the virus of inclusion blennorrhoea may be almost as common. A series of 290 cases of ophthalmia neonatorum were investigated. In 179 cases organisms only were found, in 67 both organisms and inclusion bodies, in 29 inclusion bodies only, and in 15 neither. Detailed figures are given of the distribution of incidence according to the causal organism; in general, bacteria appeared in the first 5 days and viruses in the second 5. The gonococcus causes the highest proportion of severe cases, and the diphtheroids the next highest; the inclusion bodies lie midway between these and the staphylococci.
Hugh R. Arthur.

BIOCHEMISTRY

The Value of the Guterman Test in Threatened Abortion. BENDER, S. (1947). *J. Obstet. Gynaec. Brit. Emp.*, 54, 783.

This paper is a preliminary report on 55 cases diagnosed clinically as of threatened abortion, in which the Guterman test was carried out. The test was found to provide a rapid and reliable means of differentiating those cases of threatened abortion accompanied by progesterone deficiency from those not so accompanied. Experimental work is reviewed in support of the contention that the administration of progesterone where there is no deficiency of the hormone increases the chance of the abortion progressing. The results of the present investigation support this view, although the figures are too small to be statistically significant. The limitation of progesterone therapy to cases of threatened abortion with evidence of progesterone deficiency would increase the foetal salvage rate. It is pointed out that clinically it may be difficult to distinguish between cases of threatened abortion and those of missed and complete abortion and of corpus luteum cysts. The results of pregnanediol and gonadotrophin tests simultaneously performed are of value in reaching the correct diagnosis in these cases.
R. L. Hartley.

Investigations into the Determination of Pregnanediol According to the Guterman Method. KULLANDER, S. (1948). *J. Obstet. Gynaec. Brit. Emp.*, 55, 159.

The author compares critically the methods of estimation of pregnanediol in urine. He reports on the results of 430 tests made on specimens collected from 338 patients, and arrives at the following conclusions. The variations in the day-to-day excretion of pregnanediol in non-pregnant and pregnant women are so considerable that the test should always be carefully correlated with the clinical picture, and conclusions should not be drawn from one test. In a normal menstrual cycle pregnanediol is excreted in greatest quantities in the luteal phase. The lowest excretion is during the proliferative phase. The high level associated with the luteal phase may be interpreted incorrectly as indicating the presence of cyesis, and for this reason the author believes that the Guterman test is not a reliable pregnancy test. Conflicting results have been recorded on the value of pregnanediol excretion as a guide to prognosis in threatened abortion; the author concluded that the test was unreliable for this purpose. In carcinoma of the adrenal the excretion of pregnanediol is high; in both hydatidiform mole and theca lutein cystadenomata the excretion is low. The test may be of value, therefore, in the diagnosis of these conditions. *J. Stallworthy*.

A Simplified Method for the Quantitative Determinations on Free Pregnanediol Excretion in Pregnancy. DAVIS, M. E., and FUGO, N. W. (1947). *Proc. Soc. exp. Biol. N.Y.*, 66, 39.

Over 1,500 determinations of pregnanediol excretion have been carried out on a series of about 100 women. The method is an adaptation of that described by Guterman (*J. clin. Endocrinol.*, 1945, 5, 407). The authors demonstrate the accuracy of their method by gravimetric comparisons.

Pregnancy Test using the Male Toad. MAININI, C. G. (1947). *J. clin. Endocrinol.*, 7, 653.

A new biological test for pregnancy is described. The authors inject 10 ml. of first morning urine, untreated, into the lateral lymph sac of the toad, *Bufo arenarum*. Urine (1 or 2 drops) is taken by pipette from the urinary bladder of the toad and examined microscopically. The appearance of spermatozoa 3 hours after the injection is evidence of a positive reaction.

Results of Administration of Anterior Pituitary Adrenocorticotropic Hormone to a Normal Human Subject. MASON, H. L., POWER, M. H., RYNEARSON, E. H., CIARAMELLI, L. C., LI, C. H., and EVANS, H. M. (1948). *J. clin. Endocrinol.*, 8, 1.

Pituitary adrenocorticotrophin, 25 mg., was given as a single dose to over 100 normal individuals and patients. The maximum effect was observed after 4 hours. The most pronounced change was a fall in the number of eosinophils in the normal subjects (167 to 40 per c.mm.) and in patients without Addison's disease (181 to 57 per c.mm.); this did not occur in 30 patients with Addison's disease (247 to 235 per c.mm.). The lymphocyte count also decreased, but not to the same degree, and there was a slight increase in the number of the neutrophil cells. The lymphocyte response was absent in the Addisonian cases, but the increase in the number of neutrophils was present. The eosinophil and lymphocyte response in those with Addison's disease, although absent after adrenocorticotrophin, could be elicited with 17-hydroxycorticosterone (20 mg.), but not with desoxycorticosterone and dehydrocorticosterone hemisuccinate. The very striking difference in the eosinophil response is regarded as a reliable clinical test for the adrenal-cortical reserve of 11-17-oxysteroids in Addison's disease.

H. Herxheimer.

Determination of the Relative Activities of Anti-thyroid Compounds in Man Using Radioactive Iodine. STANLEY, M. M., and ASTWOOD, E. B. (1947). *Endocrinology*, 41, 66.

The rate of iodine uptake by the thyroid was measured by giving a dose of radioactive iodine to normal volunteers and measuring the concentration in the gland. The concentration rose slowly during the period of absorption and then rapidly and parabolically to reach a constant level after 24 to 48 hours. Thirty-two compounds were thus tested in doses of 5 to 500 mg. The results obtained did not agree with the results of determinations of thyroid enlargement in rats caused by the drugs. For example, the relative activities of thiouracil, 6-*n*-propyl-thiouracil, thiourea, and 2-aminothiazole were 100, 1,100, 12, and 10 in the rat experiments and 100, 75, 100, and 250 in human studies. The results of the tests on human subjects agreed better with clinical experience, but discrepancies still remain. These are perhaps due to cumulative action, which cannot be assessed by this one-dose method of testing. *Peter C. Williams.*

Metabolic Studies in Diabetic Acidosis. II. The Effect of the Administration of Sodium Phosphate. FRANKS, M., BERRIS, R. F., KAPLAN, N. O., and MYERS, G. B. (1948). *Arch. intern. Med.*, 81, 42.

Previous workers have shown that in diabetic acidosis the inorganic phosphorus level in plasma is raised, but falls rapidly when insulin is given. This is confirmed by the authors in a series of 28 patients in whom the plasma phosphorus ranged from 4.23 to 17.2 mg. per 100 ml., and fell to less than 3 mg. per 100 ml. shortly after the start of insulin therapy. This decrease was not due to a more rapid excretion, for the phosphorus concentration in urine became subnormal, and remained so for up to 5 days, while there was a dramatic fall in plasma phosphorus in one patient with complete anuria. Since, in uncontrolled diabetes, the phosphorus concentration in urine is known to be raised, these observations suggested that in diabetic acidosis the phosphorus stores of the body are depleted, with a resulting deficiency of available phosphorus.

The authors suggest that in diabetic acidosis the lack of insulin results in a failure of phosphorylation of glucose; and so causes an accumulation of inorganic phosphorus in the plasma, and an increased excretion in the urine. They cite the observation that in alloxan diabetes in rats the onset of coma is accompanied by a decrease in the organic phosphates of the liver (chiefly adenosine diphosphate and triphosphate) and an increase in the plasma inorganic phosphorus. When insulin is given the process of phosphorylation is resumed, and for the reconstitution of organic phosphates an increased intake of phosphorus is necessary. It is suggested, therefore, that the parenteral administration of sodium phosphate should be included in the treatment of diabetic coma 4 to 8 hours after the first dose of insulin.

Wilfred E. Hunt.

Some Factors Affecting the Acidity of Urine in Man. EGGLETON, M. G. (1947). *J. Physiol.*, 106, 456.

An increase in the urine acidity (hydrogen-ion concentration) may be due not only to increased output of hydrogen ions but also to a reduction in buffering power. In experiments on 30 to 40 subjects the *p*H, ammonia concentration, and buffering power of urine were measured after the ingestion or intravenous injection of certain substances. An increased acidity of the urine accompanied by an increase in buffer output, two-thirds of which is due to phosphate, follows the ingestion of ammonium sulphate or chloride. Intravenous injections of hypertonic sucrose or sodium sulphate cause a rise in hydrogen-ion concentration and a fall in buffer output, whereas the ingestion of hypertonic urea causes a fall in hydrogen-ion concentration and a rise in buffering power. Diuresis gives rise to an increased output of buffer substances which masks the inverse relation between hydrogen-ion concentration and buffer output. The hypothesis is advanced that the increase in urine acidity, fall in buffer output, and relatively small diuresis which follow the injection of sodium sulphate and sucrose are due to secretion of antidiuretic hormone.

E. F. McCarthy.

Further Observations on the Significance of the Blood Pyruvic Acid Level in Infancy. ALLISON, E. C. (1948). *Arch. Dis. Childh.*, 23, 7.

The author thinks that the rise in blood pyruvic acid in toxaemic and haemolytic states during infancy is not associated with a deficiency of thiamine and is probably unrelated to any of the known factors in the vitamin-B complex.

The Prothrombin Response to the Parenteral Administration of Large Doses of Vitamin K in Subjects with Norman Liver Function and in Cases of Liver Disease : A Standardized Test for the Estimation of Hepatic Function. UNGER, P. N., and SHAPIRO, S. (1948). *J. clin. Invest.*, 27, 39.

The authors describe a new standardized test for the estimation of hepatic function depending on the prothrombin response to administration of large doses of vitamin K. The test was applied in 113 patients, 57 without and 56 with clinical evidence of liver disease. In the latter group 45 gave a positive response to the test, 6 a doubtful response, and 5 a negative response. The authors consider the method an important addition to the range of tests of hepatic function, since it deals with another activity of the liver. *J. W. McNee.*

Liver Involvement in Infectious Mononucleosis. EVANS, A. S. (1948). *J. clin. Invest.*, 27, 106.

Liver function tests were carried out in 19 cases of infectious mononucleosis. Seventeen of the patients had agglutinins for sheep cells in dilutions of 1 in 160 or higher, while of the 2 remaining patients, 1 with agglutinins at a titre of 1 in 80 had antibodies with the guinea-pig kidney and beef red cell absorption characteristics of the infectious mononucleosis antibody, and the other had a typical blood picture and clinical features.

The cephalin-cholesterol flocculation test was abnormal in 95% of the cases of infectious mononucleosis, and in 2 instances had altered before the heterophil antibody titre had reached a significant level. In the 2 cases in which there was a normal flocculation test, illness was mild and the patients remained ambulatory. Raised values for the thymol-turbidity test were found in 68% of cases, ranging from 4 to 10 units. Abnormalities in this test usually appeared later and were more transient than the changes in the flocculation reaction. The serum alkaline phosphatase test showed increased values in 43% of the cases, while in no case was there a significant increase in the total serum bilirubin. Serum protein determinations were made in one case of infectious mononucleosis without jaundice and in one case with jaundice, the serum having been taken about 6 weeks after the onset of the illness in each case. Marked increases in the percentages of β-globulin and γ-globulin were observed in both.

The author concludes that hepatitis occurs in many cases of infectious mononucleosis without jaundice. The cephalin-cholesterol flocculation reaction is a more sensitive indicator of this hepatic dysfunction than is the thymol-turbidity test, and may be used in differentiating such cases from cases of uncomplicated upper respiratory infection. *R. B. Lucas.*

HAEMATOLOGY

Chronic Myelogenous Leukemia. A Study of 129 Cases in which Treatment was with Radioactive Phosphorus. LAWRENCE, J. H., DOBSON, R. L., LOW-BEER, B. V. A., and BROWN, B. R. (1948). *J. Amer. med. Ass.*, 136, 672.

In this review the results, technique, and dangers of radio-phosphorus therapy are reviewed. There is considerable variation in sensitivity from patient to patient, and individual assessment of dosage is necessary so as to avoid overdosage and depression of the marrow. Life was not significantly lengthened by radio-phosphorus therapy, and at least one-third of the patients developed a terminal acute leukaemic phase. No other malignant tumours developed during the course of therapy.

Reflections on the Treatment of Acute Leucoses by Exsanguination Transfusions. (Réflexions sur le traitement des leucoses aiguës par l'exsanguino-transfusion.) BERNARD, J., and BESSIS, M. (1948). *Sang*, 19, 45.

The effects of exchange transfusion in acute leukaemia are recorded. Both clinically and haematologically dramatic temporary improvement was obtained, with subsidence of enlarged lymph glands and spleen, and a tendency towards a more normal blood and marrow picture.

Course of an Acute Leucosis Treated by Repeated Transfusions and Penicillin. (Evolution d'une leucose aiguë traitée par les transfusions répétées et le pénicilline.) MAY, E., CATTAN, R., FRUMUSAN, P., and BILSKI-PASQUIER, G. (1948). *Rev. Hémat.*, 3, 13.

The authors record the progress of a patient with acute leukaemia who was given 24 transfusions of about 150 ml. of blood during a period of 6 weeks. There was striking clinical and haematological improvement; the percentage of primitive leucocytes in the blood fell from 98% to 4% and the total leucocyte count from 40,000 to 6,400 per c.mm. The bone marrow obtained by sternal puncture was reported as normal. After a further 6 weeks the patient relapsed and died.

Remissions in Acute Leukaemia. (Les rémissions de la leucémie aiguë.) DREYFUS, B. (1948). *Rev. Hémat.*, 3, 29.

The author has reviewed the literature dealing with remission in acute leukaemia, and concludes that in almost every case this has followed blood (or plasma) transfusions. He considers the possible mechanisms by which transfusions, including exchange-transfusions, may produce a favourable effect in the first instance.

The Skin Lesions of Monocytic Leukaemia. FAIRBURN, E. A., and BURGEN, A. S. V. (1947). *Brit. J. Cancer*, 1, 352.

A case is reported with furunculosis as the first manifestation. The cutaneous lesions of 50 published cases of the Schilling type of monocytic leukaemia are then discussed and classified. Skin lesions were most frequently seen in men over 40 years of age.

Crystalline Vitamin B_{12}. RICKS, E. L., BRINK, N. G., KONIUSZY, F. R., WOOD, T. R., and FOLKERS, K. (1948). *Science*, 107, 396.

The authors describe the isolation from liver of a red crystalline substance effective in the treatment of Addisonian pernicious anaemia when given in doses as small as 3 to 150 μg. (It is possible this substance (named Vitamin B_{12}) is the purified liver anti-anaemic factor. It is not yet known whether the neurological changes in pernicious anaemia are also controlled by it.)

Activity of Vitamin B_{12} for the Growth of *Lactobacillus lactis*. SHORB, M. S. (1948). *Science*, 107, 397.

Liver extracts contain a growth factor (LLD factor) for *Lactobacillus lactis* in concentrations which closely parallel their anti-anaemic potency. The LLD factor is probably vitamin B_{12}.

Activity of Vitamin B_{12} in Addisonian Pernicious Anaemia. WEST, R. (1948). *Science*, 107, 398.

Satisfactory responses were obtained in 3 patients with Addisonian pernicious anaemia treated with simple injections of 3, 6, and 150 μg. of Vitamin B_{12} respectively.

The Reduction of Methaemoglobin in Red Blood Cells and Studies on the Cause of Idiopathic Methaemoglobinaemia. GIBSON, Q. H. (1948). *Biochem. J.*, 42, 13.

This is an important paper. Idiopathic methaemoglobinaemia appears to be caused by an inborn deficiency of coenzyme factor I, a link in the chain of reactions whose effect is to prevent the oxidation of haemoglobin to methaemoglobin. The reducing action of ascorbic acid helps to prevent the complete oxidation of haemoglobin to methaemoglobin. Methylene blue is effective in treatment but reduces methaemoglobin by another enzymic pathway.

Refractory Iron-Deficiency Anaemia Treated with Intravenous Saccharated Oxide of Iron. DAVIDSON, L. S. P., and GIRDWOOD, R. H. (1948). *Brit. med. J.*, I, 733.

A good haematological response was obtained when a patient was treated with intravenous iron according to the method of Nissim (*Lancet*, 1947, 2, 49), but there were some toxic reactions.

Chronic Haemolytic Anaemia with Haemoglobinuria. The Marchiafava-Micheli Syndrome. HICKEY, M. D., and MALLEY, L. K. (1948). *Quart. J. Med.*, 27, 1.

The authors describe observations made on a patient observed for 3 years. Haemoglobinuria appeared to be precipitated on four occasions following iron therapy and once following blood transfusion. Normal human serum heated to 56° C. had some inhibiting effect on haemolysis both *in vitro* and *in vivo*.

Studies on the Pancytopenia of Kala-Azar. CARTWRIGHT, G. E., CHUNG, H. L., and CHANG, A. (1948). *Blood*, 3, 249.

This is an important contribution to the haematology of kala-azar. The bone marrows of 27 patients were examined. There was general hyperplasia with increase in reticulo-endothelial cells. It is suggested that the anaemia, leucopenia, and thrombocytopenia are consequent on a disturbance in cell development, perhaps due to "hypersplenism," and are not simply due to a crowding out of the marrow cells by hyperplasia of the reticulum.

Studies on the Conglutination Test in Erythroblastosis Foetalis. WIENER, A. S., and GORDON, E. B. (1948). *J. Lab. clin. Med.*, 33, 181.

The authors compared several techniques in the demonstration of Rh antibodies in the sera of pregnant Rh-negative women. The sensitivity of the tests in ascending order were: (1) the blocking test (the least satisfactory), (2) the plasma conglutinin and antiglobulin tests (Coombs' test), and (3) the albumin-plasma method (the most sensitive). Incomplete antibodies seem to pass through the placenta readily, but complete antibodies generally do not appear to do so.

Physiological Jaundice of the Newborn. Some New Measurement of the Factors Concerned. MOLLISON, P. L. (1948). *Lancet*, 1, 513.

This is an important contribution to the understanding of the physiological jaundice of the newborn. Using the method of differential agglutination, transfusion studies carried out with placental and adult corpuscles demonstrated that a proportion of the infant's corpuscles are broken down at about twice the adult rate during the

first 10 days of life. There is thus some indirect evidence of an increased rate of haemoglobin catabolism in the neonatal period. Previous work and the author's own observations of impaired bromsulphalein excretion by the infant's liver suggest, however, that inefficiency of the liver is the most likely cause of neonatal jaundice.

Congenital Hemolytic Jaundice. The Pathogenesis of the "Hemolytic Crisis." OWREN, P. A. (1948). *Blood*, 3, 231.

The author demonstrates how "anaemic" crises may be produced during the course of congenital haemolytic jaundice. Six patients were studied and a temporary depression of erythropoiesis was found. Serial bone marrow studies demonstrated how restoration of erythrocyte formation preceded clinical recovery. These observations are of great interest and prompt the question as to how many of the so-called "haemolytic crises" previously reported have a similar explanation.

Normal Red-cell Survival in Men and Women. CALLENDER, S. T., POWELL, E. O., and WITTS, L. J. (1947). *J. Path. Bact.*, 59, 519.

The survival of transfused normal blood was contrasted in 2 men and 4 women. In the men the elimination curve was linear; in the women it was appreciably curved. In both, elimination was complete in about 120 days. The average life of the transfused corpuscles was 63 days in the men and 54 days in the women. Blood loss due to menstruation is thought partly to explain the differences between the two sexes.

Blood Volume in Pregnancy. A Critical Review and Preliminary Report of Results with a New Technique. McLENNAN, C. E., and THOUIN, L. G. (1948). *Amer. J. Obstet. Gynec.*, 55, 189.

The authors studied the blood volumes of 20 pregnant women at term and at 7 weeks post partum, and 10 non-pregnant controls. They used Evans blue dye. The total blood volumes and plasma volumes were significantly greater in the pregnant women, but individual variation was great and many more determinations need to be done before a reliable picture is obtained.

A Method for Obtaining Bone Marrow by Vertebral Spinous Process Puncture. HUSS, J. H., GILBERT, J., and LIEBOW, A. A. (1948). *Yale J. Biol. Med.*, 20, 291.

The spines of the lower thoracic and upper lumbar vertebrae may be punctured safely and readily, and marrow is withdrawn similar in composition to sternal marrow. This method is also described by Loge (*Blood*, 1948, 3, 198); it is probably safer and less alarming to the patient than is sternal puncture.

Study of Fixed Tissue Sections of Sternal Bone Marrow Obtained by Needle Aspiration. I. Method and the Morphology in Various Conditions. II. Comparison of Nucleated Cell Count and Volumetric Pattern with Histologic Appearance. WEISBERGER, A. S., and HEINLE, R. W. (1948). *Amer. J. med. Sci.*, 215, 170.

In this report are described a technique for sectioning marrow particles obtained by aspiration and a comparison between the total nucleated cell count, the volumetric pattern as determined by haematocrit, and the histological appearance of the marrows of 60 patients.

Clinical Value of Some Methods of Estimating Erythrocyte Sedimentation Rate. SINTON, J. R. (1948). *Brit. med. J.*, 1, 391.

A comparison was made between the erythrocyte sedimentation rates (Westergren and Wintrobe techniques) of 61 female patients with pulmonary tuberculosis and the clinical course of the disease. The corrections of Wintrobe and of Whitby and Hynes for anaemia were applied. The patients, whose ages ranged from 14 to 46 years, were not specially selected, and were under continuous observation for periods ranging from 6 weeks to 6 months.

The author concludes that since the Westergren method gives figures that "agree better" with the clinical course than do those obtained by either of the methods involving correction, it is the most useful in assessing the degree of activity of the disease and the direction of its progress. *Wilfred E. Hunt.*

Erythrocyte Sedimentation Rate. The Effect of Alcohol as Contaminant. SYKES, W. O. (1948). *Brit. med. J.*, 1, 393.

The rate of sedimentation is diminished if blood is contaminated with alcohol in concentrations of 1% or more.

MORBID ANATOMY AND HISTOLOGY

Phase-contrast Microscopy: Applications to Pathological Histology and Blood Cytology. (L'examen microscopique par les "contrastes de phases." Ses applications à l'histologie pathologique et à la cytologie sanguine.) FEISSLY, R., and QUÉBATTE, R. (1947). *Rev. Hémat.*, 2, 411.

A simple explanation of the principles of phase-contrast microscopy, quite the most elementary which the abstracter has yet seen, and well worth studying. *G. Discombe.*

Sludged Blood. KNISELY, M. H., BLOCH, E. H., ELIOT, T. S., and WARNER, L. (1947). *Science*, 106, 431.

Histological observations were made on living animals and men; the authors claim that these lead to a more precise understanding of a variety of mechanisms whereby injuries and diseases damage the human body. In 600 non-anaesthetized human patients suffering from a wide variety of diseases the blood cells were seen to be agglutinated into masses (not rouleaux). This appeared to change the blood from its normal relatively fluid state to a circulating sludge. The authors believe that: (1) the resistance of sludged blood to its own passage through the bottle-necks of the circulatory system reduces the rate of blood flow through all the open vessels of the body; (2) agglutinated red cells are ingested and destroyed by the phagocytic cells of the liver and spleen; (3) there is settling and sedimenting of masses of agglutinated blood cells out of the moving blood plasma during life; (4) various degrees of reduction in circulating blood volume cause intermittent, prolonged, controlled shutting off of the arterioles in a selected series of tissues and organs.

The authors studied sludges in the conjunctival vessels in infective and traumatic conditions, hypertension, embolic states, hysteria, alcoholism, and normal uncomplicated pregnancy. Although it is not suggested that the sludges explain the clinical features of diseases in which they are found, they are obviously of importance in embolic conditions. The authors hope that it will become possible to keep the blood in a fluid and non-agglutinated state with intact vessel walls in conditions in which sludges have been observed.

R. Winston Evans.

Primary Systemic Amyloidosis. IVERSON, L., and MORRISON, A. B. (1948). *Arch. Path.*, 45, 1.

Two cases of atypical, or primary, amyloidosis are reported in both of which the heart was extensively involved and many other tissues affected. Difficulties in the classification of amyloid disease and the staining properties of the substance in different circumstances are discussed.

Histological Changes in the Small Intestine in Disturbances of Fat Absorption. (Xanthomatosis of the Small Intestine.) (Über die histopathologischen Veränderungen am Dünndarm bei Störungen der Fettresorption (sog. Xanthomatose des Dünndarms).) FREI, R. (1947). *Schweiz. Z. Path. Bakt.*, 10, 685.

Two cases of xanthomatosis of the intestine are reported. A woman of 84 died of cerebral arteriosclerosis, apparently without having shown signs of gastro-intestinal upset during life. At necropsy the small gut showed yellowish discoloration of the rugae, and there was ulcerative colitis. Microscopically there was an extensive xanthomatosis of the submucous coat of the small intestine without signs of inflammation, and fulminating colitis was present in the large gut. The second patient, a man of 43, died after a sprue-like illness of several months' duration. On microscopical examination large foam cells were seen in the submucosa of the entire gut; in addition the lymph nodes of the mesentery showed fatty infiltration and a granulomatous xanthosis. *R. Salm.*

The Adrenal Cortex in Essential and Renal Hypertension. FISHER, J. A., and HEWER, T. F. (1947). *J. Path. Bact.*, 59, 605.

Material was taken from routine necropsies on 55 subjects with essential hypertension, 15 with renal hypertension, and 57 controls. A diagnosis of hypertension was based on the presence of two out of three positive criteria: a high blood pressure record, left ventricular hypertrophy (measured by heart weight), and hypertensive arteriolar changes. In assessing heart weight the criteria of Moritz and Oldt were applied. Heart weights of 450 g. in males or 350 g. in females were taken to indicate hypertension, and those weighing less than 400 g. in males and 300 g. in females to denote absence of hypertension. Blood-pressure readings of 160/90 mm. Hg or more were considered to indicate hypertension at any age, and no case was accepted as a control if there was a record of a systolic pressure of over 140 mm. or a diastolic over 90 mm. Hg.

Formalin-fixed adrenals were weighed and portions analysed for percentage lipid. Frozen sections were stained with Scharlach R and examined under polarized light. The content of sudanophil lipid and anisotropic lipid in the three equal zones of the cortex (outer, middle, and inner thirds) was recorded in terms of arbitrary units: 0, $\pm$, 1, 2, or 3. Adding the values for the three zones gave a value for lipid content; $\pm$, indicating a trace, was taken as $\frac{1}{4}$. A significant association was shown to exist between essential hypertension and each

of the following: cortical nodularity, percentage lipid (estimated chemically), cortical sudanophil lipid, and cortical anisotropic lipid.

The authors take the view that increased cortical lipid is an indication of altered endocrine activity. They suggest that their findings may all reflect variation in a single normal process by which the adrenal cortex maintains glomerular filtration, for example, by increasing the efferent glomerular arteriolar tone.   *F. A. Langley.*

The Relationship Between Rheumatic Carditis and Subacute Bacterial Endocarditis.   MacILWAINE, Y. (1947). *J. Path. Bact.*, 59, 557.

Aschoff bodies in the myocardium of patients with infective endocarditis have been noted in many reports in the literature. The author reviews the evidence on their significance, and concludes that they do not represent a reaction to the bacterial infection, but that their presence is evidence of a rheumatic carditis. In the work here recorded, 34 cases of subacute, and 12 cases of acute, bacterial endocarditis were studied histologically; in every case in which Aschoff nodes were present an attempt was made to estimate their age. Histological criteria are given for the division into: under 2 months, 2 to 4 months, 4 to 6 months, and over 6 months. In the subacute group, 29 had Aschoff nodes, 5 did not; of the acute cases 7 had Aschoff nodes, 5 did not. In many cases crops of rheumatic lesions of 2 or even 3 different age periods were present. The estimated age of the Aschoff bodies corresponded to the clinical duration of the bacterial endocarditis in 64.7% of the subacute and in 25% of the acute cases. The author concludes that in the majority of cases of subacute, and in a smaller percentage of cases of acute, bacterial endocarditis the bacterial lesion is superimposed upon the site of an active rheumatic carditis.

Liver Disease in Johannesburg.   Relation to Pellagra.   GILLMAN, J., and GILLMAN, T. (1948). *Lancet,* 1, 169.

The authors found that only 33 (12.6%) out of 261 Africans who died accidentally had normal livers. By contrast in a control group of 90 Europeans who died accidentally 61 (67.8%) had normal livers. The lesions in most of the abnormal livers in Africans could be classified on histological grounds into the four types already described by these workers as occurring among pellagrins. In these types the deposition of large quantities of iron is the chief feature and represents a profound metabolic disturbance. It is suggested that the large inert iron-containing molecules may complicate the primary metabolic disorder associated with liver disease, and thus prepare the background for the development of premature arteriosclerosis, cirrhosis of the liver, keloid formation, and reticulosis, all of which are known to be prevalent among Africans.

Christopher Hardwick.

Hepatic Alkaline Phosphatase: Histological and Microchemical Studies on Liver Tissue in Normal Subjects and in Liver and in Bone Disease.   SHERLOCK, S., and WALSHE, V. (1947). *J. Path. Bact.*, 59, 615.

The histological distribution of alkaline phosphatase in portions of liver obtained by aspiration biopsy (in many cases from serial aspirations) was studied by a modification of Gomori's technique. Increase in the enzyme in liver-cells and walls of sinusoids was invariable in acute hepatitis; other variations characterized obstructive jaundice and cirrhosis.

Automatism of the Nephron and its Histological, Physiological, and Pathological Aspects.   (Automatismo nefronico e suoi aspetti isto-fisiopatologici.)   MONTALDO, G. (1947). *Arch. ital. Anat. Istol. pat.*, 20, 45.

Studies were made on the structure of the juxtaglomerular apparatus of the human kidney in health and disease, and on the anatomical and physiological relation of the glomerulus and tubule. The author advances the conception of "automatism of the nephron," basing his theory on the peculiarities of structure of the afferent arteriole, the epithelioid cells in the wall of the distal end of which are continuous with the special preglomerular cells of Zimmermann's cushion. The latter tissue fills the triangular space between the afferent and efferent vessels and the intermediate segment of the tubule (that portion joining the ascending limb of Henle's loop to the second convoluted tubule). It is continuous on one side with the ampulla and on the other is, of course, contiguous with the macula densa. Thus Zimmerman's "Polkissen" forms a bridge between these different parts of the nephron, and evidence is adduced that this tissue is sensitive both to pressure and to chemical stimuli. It is thought to be sensitive to the osmotic pressure of the fluid in the intermediate tubular segment, and swells or shrinks according to the degree of dilution or concentration of this fluid. When the urine is dilute, swelling of the cushion causes the glomerulus to contract in systole, with a consequent slowing in the rate of filtration and in the rate of passage of the filtrate through the tubule; and thus the concentration of the urine rises. The opposite also occurs, and the relations of filtration and absorption can be expressed algebraically. In this way automatic self-regulation is established in each single nephron, although the rate of filtration also remains dependent on such general factors as blood pressure, hormonal and nervous stimuli, and drugs. Moellendorf's "nephronic index" relates the size of the glomerulus to its corresponding tubule. *In vivo* this relation may vary between wide limits, and the author suggests the term "dynamic nephronic index" to relate the powers of concentration and dilution of the nephron. Those diseases of the kidney most typically associated with disturbance of the power of concentration (nephrosclerosis, chronic diffuse glomerulonephritis) show the most markedly selective changes in the self-regulating tissue of the nephron. When hyposthenuria or isosthenuria is present there is always pronounced alteration in the afferent arteriole, cushion, and ampulla of the surviving glomeruli. These glomeruli can no longer be influenced by the state of the intratubular fluid, and the filtration rate depends on the degree of patency of the afferent vessel and on the systemic blood pressure.

E. G. Sita Lumsden.

Intestinal Ulceration Due to Arterial Necrosis (Malignant Hypertension and Polyarteritis Nodosa).   LE NAVASQUEZ, S., and FRENCH, E. B. (1947). *Guy's Hosp. Rep.*, 96, 85.

Clinical and post-mortem records are reported of 2 patients, one with malignant hypertension and the other with polyarteritis nodosa, both presenting a clinical picture of predominantly intestinal disease. The first patient had a short history of abdominal pain and bloody diarrhoea, the second a more prolonged story of anaemia and steatorrhoea. The most striking pathological finding in both cases was the presence of extensive ulceration due to necrotizing arteritis of the small intestine. The histological appearances of the arterial lesions in

both cases were very similar. In the case of polyarteritis nodosa there was also complete disorganization of the mesenteric lymph nodes, which were the seat of extensive ischaemic necrosis and replacement fibrosis; this, together with the ulcerative destruction of the intestinal mucosa, was the cause of the sprue-like syndrome which was present during life. Another interesting feature of this case was the persistent lymphopenia (25 to 1,250 lymphocytes per c.mm.), accounted for at necropsy by the finding of extensive ischaemic necrosis of the splenic lymphoid tissue and by the above-mentioned mesenteric lymph-node destruction. *John R. Forbes.*

Massive Neoplastic Embolism. TILL, A. S., and FAIRBURN, E. A. (1947). *Brit. J. Surg.*, 35, 86.

A tumour mass which had clearly originated in a pulmonary carcinoma was found in the common femoral artery, extending into the profunda artery with surrounding blood clot blocking these arteries and the superficial femoral artery.

The Nature of So-called Myoblastomata. (Die Natur der sog. Myoblastentumoren.) WEGELIN, C. (1947). *Schweiz. Z. Path. Bakt.*, 10, 631.

Details of 10 cases of myoblastoma are given, the tumour being situated in gums, tongue, wrist muscles, and skin. The author thinks the growths are derived from undifferentiated mesenchymal cells and not from muscle-elements and that they are capable of storing proteins and lipids.

Cancer of the Stomach in Addison's Anaemia. BOURNE, W. A. (1948). *Brit. med. J.*, 1, 92.

The author investigated 15 unselected cases of pernicious anaemia, discovering 3 cases of carcinoma of the stomach and 1 of leiomyoma. In all 3 cases of carcinoma the neoplasm was in the lower third of the stomach, and radiology showed narrowing. This may be the first indication of carcinomatous change, or it may be a general characteristic of these stomachs. It is not productive of delay. The lower stomach is not usually abnormal in pernicious anaemia, and gastroscopy confirms this. The number of cases is too small for conclusions to be drawn, but the author suggests that in cases of pernicious anaemia an abnormal mucosa in the prepyloric region should be viewed with suspicion.

Intracranial Teratomas and Teratoid Tumors. (In English.) MULLER, R., and WOHLFART, G. (1947). *Acta psychiat., Kbh.*, 22, 69.

In 8 cases of the rare teratoid form of cerebral tumour, 5 tumours were situated in the region of the pineal gland, 1 was in the third ventricle, 1 in the suprasellar fossa, and 1 in the cerebellum. Histological examination of the tumours revealed the presence of ectodermal and mesodermal structures in every case, but endodermal tissue was found in only 3 cases.

Carcinoma Ovarii and Cerebellar Degeneration. BROUWER, B., and SCHLESINGER, F. G. (1947). *Proc. K. Akad. Wet., Amst.*, 50, 1329.

A patient, aged 51, with ovarian carcinoma, began to show cerebellar symptoms about 15 months before death. Psychical disturbances were present at the beginning but regressed. As in other reported cases the cerebrospinal fluid contained an excess of protein and gave colloidal reactions of mild paretic type. Post-mortem examination revealed carcinoma of the left ovary and adnexa with metastases in the neighbouring part of the abdominal cavity. The cerebellum showed no macroscopical evidence of disease, but microscopically there was complete disappearance of Purkinje cells, with relative preservation of baskets and almost complete integrity of the granular layer. The left dentate nucleus was severely degenerated, as were the ventral part of the right nucleus and to a minor degree the roof nuclei. No degeneration of inferior olives was seen. There was loss of many cells in the vestibular nuclei. Meningeal and perivascular infiltration in relation to the degenerated areas of the cerebellum and brain stem was present. The authors consider that the metabolic disturbances associated with carcinoma affect parts of the central nervous system which are predisposed to degeneration by abiotrophy in Gowers' sense of the word. *J. G. Greenfield.*

An Unusual Case of Adrenal Carcinoma with a Note on the Application of a New Colour Test. BROSTER, L. R., and PATTERSON, J. (1948). *Brit. med. J.*, 1, 781.

A girl aged 14½ years had a history of increasing virilism for 18 months, and of fits followed by coma for 4. On admission she had right spastic hemiplegia (which passed in 3 days), bilateral papilloedema, hypoglycaemia, and a large left hypochondriac tumour. Radiographs showed calcified streaks in a large adrenal shadow and premature epiphysial fusion corresponding to 20 years or over. The 17-ketosteroid excretion was 1,980 mg. daily and the dehydro*iso*androsterone test (Patterson) was strongly positive. Death occurred a fortnight after admission, and 21 months after the onset of the virilism. At necropsy the left adrenal tumour together with the attached kidney weighed 6½ lb.; microscopically the structure was that of a malignant adrenal cortical tumour, although mitotic figures were scarce. No significant changes were found in other organs and there were no secondaries. Patterson's urinary colour test (*Lancet*, 1947, 2, 580) was used to differentiate carcinoma from hyperplasia. *Henry Cohen.*

Tumors of the Carotid Body. LeCOMPTE, P. M. (1948). *Amer. J. Path.*, 24, 305.

This is a useful description of the characters of carotid body tumours, based on a personal study of 17 surgically removed specimens. No true chromaffin reaction was demonstrated in any of these, and no evidence of the secretion of adrenaline was obtained by assay of the fresh tissue in two instances. These findings thus support the other evidence that the carotid body is not part of the chromaffin system, but is a chemoreceptor. *R. A. Willis.*

Chemical Character of the Enterochromaffin Cells. GOMORI, G. (1948). *Arch. Path.*, 45, 48.

The author discusses the histochemical reactions of the enterochromaffin cells and describes a new staining method (methenamine-silver) which is applicable to formaldehyde-fixed tissues. By using the Coujard technique he was able to show that the typical histochemical reactions of the granules are due to the presence of a resorcinol derivative. He has disproved the ideas of Lison and Cordier that a catechol compound exists, and he is unable to confirm the contention of Jacobson that a pteridine compound and desoxyribose are present in the cells. *E. T. Ruston.*

Papillary Carcinoma of the Thyroid and Lateral Cervical Region. So called "Lateral - Aberrant- Thyroid." CRILE, G. (1947). *Surg. Gynec. Obstet.*, 85, 757.

A primary tumour was found in the thyroid gland itself in all of 16 consecutive cases in which the thyroid gland was explored or a lobe removed. In 4 of these the lesion was bilateral, but in none was more than a single nodule found in a lobe. The author reviews the difficult problem of the relationship of these papillary tumours to carcinoma of the thyroid in the light of personal experience.

Pathologists who have long taken the view that the lateral cervical masses were metastases of an unusually low degree of malignancy are thereby justified. Moreover it appears that the primary may remain small and easily escape detection from its frequent position in the postero-medial part of the lobe.

Lingual Goiter. Report of Three Cases. GOETSCH, E. (1948). *Ann. Surg.*, 127, 291.

Three cases are described of hypertrophic thyroid tissue at the base of the tongue in non-migration of the thyroid anlage from the region of the foramen caecum. Each patient showed evidence of mild hyperthyroidism, and the histological structure of all three resected specimens was very similar to that of foetal adenoma of the cervical thyroid. It is pointed out that the majority of lingual thyroid swellings are goitres. These are benign, and carcinoma has been reported in only rare instances, always in men. Some pharyngeal and laryngeal obstruction is usually the indication for operation, and partial removal by the buccal route is the treatment of choice. Thyroid insufficiency is the rule after operation.

Anatomical and Clinical Study of Spontaneous Para-thyroid Tetany in Adults. (Étude anatomo-clinique des tétanies parathyroïdiennes spontanées de l'adulte.) LEVRAT, M., and BRETTE, R. (1947). *Ann. Endocrinol., Paris*, 8, 117.

Spontaneous parathyroid tetany (S.P.T.) has often been described in children, but only rarely in adults. A woman of 56 was treated for heart failure due to hypertension. She improved temporarily and remained under observation until her death 8 months later. Four months after her first admission she had an attack of S.P.T. which responded to calcium and to parathyroid extract, and best to combined calcium and vitamin-D treatment. She had had such attacks during the previous 2 years. Radiologically, cervical calcifications the size of a bean, which moved on swallowing, were found in the lateral thyroid regions. Necropsy showed the calcifications at the site of the parathyroids. Histologically, the parathyroids showed marked central sclerosis with calcium deposits and perivascular haemorrhage. At the periphery, parathyroid tissue was seen to be divided and encircled by fibrous tissue, as in cirrhosis of the liver.

Sarcoid. FREUDENTHAL, W. (1948). *Brit. J. Tuberc.*, 42, 11.

This paper records evidence in favour of the belief that Boeck's sarcoid is a manifestation of tuberculosis. Certain links exist between the two diseases which do not, however, prove the hypothesis. Thus, coexistence occasionally occurs, skin sarcoid lesions have been known to change into lupus and *vice versa*, and terminal change of sarcoid into tuberculosis has been demonstrated at necropsy more than once. The chief factors against a tuberculous aetiology have been the histological picture, the negative tuberculin reaction, and the absence of tubercle bacilli. The author demonstrates sarcoid tissue in histological sections of lupus, and points out that it is formed in moderate amount during treatment with calciferol. Even more frequently, lupus features are seen in sarcoid. Lesions of spontaneous tuberculosis in some animals consist chiefly of epithelioid tissue, and in rats and dogs the tuberculin reaction remains negative. Wells and Wylie at Oxford have found that sarcoid serum actively neutralizes tuberculin. Tubercle bacilli have now been isolated from about 26 documented cases of sarcoid, and there is reason to believe that, as in tuberculides, bacilli can be demonstrated only at the earliest stage of the lesion. The newest finding in favour of a tuberculous origin is that sarcoid (presumably in skin) improves with calciferol therapy.   *T. Semple.*

Primary Atypical Pneumonia. Report of Eight Cases with Autopsies. PARKER, F., JOLLIFFE, L. S., and FINLAND, M. (1947). *Arch. Path.*, 44, 581.

Death in primary atypical pneumonia is uncommon. In the present 8 cases necropsy revealed congestion of the air passages and lungs, basal collapse with apical emphysema, and small grey pneumonic foci. Histologically, the alveolar exudate was "mononuclear"; there were proliferation and desquamation of alveolar epithelium and infiltration of septa and bronchioles. "Alveolar membranes" were present in half the cases. Many small arteries and veins were thrombosed. The lungs also showed changes due to secondary infection, such as pus in the bronchi. In the liver there were small focal necroses and in the brain, especially in one case, perivascular haemorrhages with glial proliferation. The appearances resembled those of psittacosis. In one case there were psittacosis antibodies, but this was probably fortuitous. Attempts to isolate a virus and demonstrate inclusion bodies were unsuccessful.

D. M. Pryce.

The Pathogenesis of Congenital Polycystic Lung and its Correlation with Polycystic Disease of other Epithelial Organs. Reconstruction of Cystic Elements in Two Cases. NORRIS, R. F., and TYSON, R. M. (1947). *Amer. J. Path.*, 23, 1075.

The authors have written a series of articles on cystic disease in numerous organs and they discuss its pathogenesis, which might be a degenerative process leading to isolation and cyst formation, and may also involve the question of deficiencies of the circulation at the site of the cystic change. Two cases are here described of congenital cysts of a lung. Detailed anatomical descriptions showing lack of communication with the bronchi are given.

Pathology of Skeletal Muscle. (Zur Pathologie der Skelettmuskulatur.) HEDINGER, C. (1948). *Schweiz. med. Wschr.*, 78, 145.

Two cases of damage to skeletal muscle after carbon monoxide poisoning are reported. In the first a contracture of the hand, of Volkmann type, took place; in the second a painful swelling of the gastrocnemius proceeded to calcification. Both cases were in young adults.

The author was able to trace 28 similar cases in the literature, and outlines the following entity. After severe

carbon monoxide poisoning there is transient albuminuria (rarely glycosuria), and in those parts of the body which have been exposed to pressure a triad may develop, consisting of skin damage and loss of sensibility and motility. The former resembles a burn. The sensibility is reduced distal to the skin lesions. Involvement of the muscles is shown by paralysis, swelling, and pain, and leads to permanent damage—paralysis, atrophy, contracture, calcification. Infection of gangrenous skin and underlying muscles may occur, with subsequent death. Biopsies show initially hyaline, fatty, or waxy degeneration of muscle, with some inflammatory reaction and occasional haemorrhage, and calcification and scar formation in the later stages.

Twenty-one Observations on Muscular Dystrophy in Hyperthyroidism. (Vingt et une observations de dystrophie musculaire hyperthyroïdienne.) FROMENT, R., GUINET, P., DEVIC, MME. M., and DEVIC, M. (1947). *Bull. Soc. méd. Hôp. Paris,* 63, 843.

Of 21 cases investigated all except one were in women; in 16 toxic signs had existed for months, in the remainder for years. Weakness and difficulty in rising from a kneeling position were the chief muscular symptoms; wasting was found in most of the lower limb extensors and much less often in the arm muscles. Biopsies carried out in 3 cases revealed changes similar to those found in congenital myopathies. Creatinuria was present in 5 cases. Where the treatment of the hyperthyroidism was successful, the muscular dystrophy disappeared or was relieved, in one case after only 2 days but in most cases after months. Administration of iodine and aminothiazole, radium therapy, and thyroidectomy were the forms of treatment used. The authors speculate on whether the wasting is due directly to the thyroid state, or whether both run parallel for some unknown cause. Similar effects are seen in the muscles of animals after injection of thyroid extract. It is also emphasized that the two opposing states of thyrotoxicosis and myxoedema both produce muscular changes, though these are quite different in the two conditions; this suggests that the cause may lie in the thyroid itself. *T. E. C. Early.*

Talcum Powder Granuloma: A Frequent and Serious Postoperative Complication. EISEMAN, B., SEELIG, M. G., and WOMACK, N. A. (1947). *Ann. Surg.,* 126, 820.

Thirty-seven cases of post-operative granulomata giving rise to clinical complications and proved due to talcum powder are described. It is stated that 22.6% of all gloves have perforations after use at operation. The risk of this post-operative complication is therefore considerable. Clinically the condition presents itself as a non-healing wound, a faecal fistula, intestinal adhesions, an obstruction or stenosis, or as a tumour mass. Talc produces pathologically the same kind of massive fibrous reaction as pulmonary silicosis, except that in the case of the granulomata the reaction is more localized. The

usual histological picture of small round cells and multinucleated giant cells makes the resemblance to a tuberculoma obvious. The authors stress the danger of using talc and recommend that it be banned from surgery. Alternatives are discussed, the best proposed to date being potassium bitartrate.

Actinomycosis of the Brain. LEWIN, W., and MORGAN, A. D. (1947). *J. Neurol. Neurosurg. Psychiat.,* 10, 163.

A case of actinomycosis apparently had its origin in the sphenoid bone, and also affecting the right cavernous, the superior longitudinal, the right sigmoid, and the superior petrosal sinuses, with extension to the right atlanto-occipital joint and the exterior of the right cerebral hemisphere. The patient was a man aged 31 years, and 5 months elapsed between the onset of symptoms and the necropsy, at which actinomyces were identified for the first time. Ventriculograms were normal, but there were significant alterations in the withdrawn fluid. From an abscess on the right side of the neck a non-haemolytic *Staphylococcus aureus* was grown. There was no evidence to show the route whereby the actinomyces reached the sphenoid. This is only the fourth recorded case of primary actinomycotic osteomyelitis of the sphenoid bone. *Zachary Cope.*

Studies in the Biology of the Cervix and its Relation to Puerperal Infections. McILRATH, M. B., and HELLESTRAND, A. L. (1947). *J. Obstet. Gynaec. Brit. Emp.,* 54, 746.

The authors feel justified in making the general conclusions that cervical lesions constitute a danger to the mother during pregnancy and increase her liability to develop low-grade pelvic infection after confinement. The added factor of cervical trauma does not seem to be of significance among primigravidae. On the other hand, among abnormal multigravidae the presence of pre-existing trauma and injury during labour does seem to aggravate a tendency to develop inflammatory conditions and to encourage spread to the uterus and adnexae. As a prophylactic measure, routine antenatal inspection of the cervix is recommended. When abnormal conditions are discovered the most satisfactory treatment appears to be the application of penicillin-sulphonamide powder.
[There are some good descriptions and illustrations of the histological changes which occur in the cervix during pregnancy.] *R. L. Hartley.*

Classification on the Histologic Reactions in Allergic Diseases. BOHROD, M. G. (1947). *Amer. J. Med.,* 3, 511.

The author classifies the histological appearances of allergic reaction. Anatomically there are three main varieties: (1) necrotizing, which may be either organ-selective or cell-selective; (2) anaphylactoid; (3) granulomatous, which may be either tuberculoid or rheumatoid.

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AUTHORS OF ARTICLES ABSTRACTED